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PHYSICAL METHODS IN QUALITATIVE AND QUANTITATIVE CHEMICAL ANALYSIS

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During the first half of the present century one has witnessed a great expansion in the role which the physicist plays in the field of chemical analysis.

The instruments and techniques developed by the physicist for the determination of physical constants have furnished the chemical analyst with new devices which can be used for the quantitative and qualitative determination of the elementary composition of substances. These new methods have supplemented the classical methods of gravimetric and volumetric analysis which experienced their greatest growth during the nineteenth century. The physical methods have enabled the analyst to broaden the scope of analysis, since in many cases accurate measurements can be made without destruction of the sample. He is also able to analyze complex mixtures quantitatively, which previously would have presented almost unsurmountable difficulties. The analyst now has at his disposal physical methods which enable him to investigate problems of structure in organic chemistry, reaction kinetics, and even the biochemistry of living cells.

By absorption spectroscopy the analyst is able to identify components and to measure the amounts present in certain compounds or mixtures of substances. In absorption spectrophotometry use is made of the property of the absorption of electro-magnetic radiation by substances throughout the spectrum extending from the infrared region through the visible, ultra-violet, and x-ray portions of the spectrum to the gamma ray region. In each region of the spectrum appropriate instrumentation is necessary for the detection of that particular type of radiation.

Emission spectroscopy, while known for many years, has only recently acquired widespread use in providing rapid quantitative analyses. A new epoch in spectrochemical analysis occurred approximately fifteen or twenty years ago when high quality spectrographic equipment began to appear in many industrial laboratories. Previous to this time such equipment was found only in the research laboratories of the universities and the government. Bunsen and Kirchhoff in 1860 first used the spectroscope in performing a chemical analysis. The foundations for qualitative emission spectroscopy, however, were laid by W. Talbot in 1826, when he observed that the radiations, emitted by salts of potash and sodium in flames, experienced a definite refraction when passed through a glass prism.

Certain compounds with a special structure are capable of absorbing radiation at particular frequencies and re-emitting the energy at lower frequencies. Such substances are said to possess the property of fluorescence. This fluorescence radiation can be used in quantitative analysis, since the intensity of the fluorescence is proportional to the concentration in dilute solutions. The red fluorescence so apparent in chlorophyll solutions was first observed by Brewster in 1833. Sir G. G. Stokes in 1852 was able to show from his studies of the fluorescence that chlorophyll

consisted of a mixture of two pigments. The spectroscopic equipment of Stokes consisted of a glass prism used with the naked eye.

The mass spectrograph is another instrument developed by the physicist which has recently found a place in the field of chemical analysis, especially in the laboratories of the petroleum and rubber industries. In the quantitative investigation of reactions where stable isotopes are used as tracers, the mass spectrograph is the indispensable instrument.

Recently a tool which the physicist has furnished the chemical analyst is in the field of radioactivity. The analyst is able with the proper equipment to follow in a quantitative fashion chemical reactions which could not be traced by any other method. The most recent addition to the field of quantitative analysis is the use of neutrons. The development which this field received during the recent war period has opened new areas in analytical work the extent of which cannot be predicted.

A symposium on "Physical Methods in Qualitative and Quantitative Analysis" was held under the joint sponsorship of Section F of the Ohio Academy of Science and the Ohio Section of the American Physical Society at Miami University, Oxford, Ohio, April 6, 1951. Its purpose was to bring together the research men from the industrial and academic laboratories to consider the progress that has been made in a borderland field in which the results of physical research have played an important part. The following six papers were presented as a part of the symposium: "Emission Spectroscopy in the Steel Industry" by E. R. Vance; "Absorption Spectrophotometry" by D. L. Timma; "Analysis Using Infrared Spectroscopic Methods" by Robert A. Oetjen; "Chemical Analysis by X-Rays" by C. H. Shaw; "Analytical Applications of Mass Spectrometry" by D. R. Lawler; "Radioactive Methods of Analysis" by G. D. Calkins.

EMISSION SPECTROSCOPY IN THE STEEL INDUSTRY

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Large scale production of high grade electric furnace steels demands rapid, accurate analytical control. Without exaggeration it might be said that the analytical laboratory controls the steel industry—it is impossible to make good steel without close chemical control.

To produce a heat of alloy steel, the furnace is charged with steel scrap or pig iron. After the melting period, when the molten bath has been thoroughly mixed, a sample is cast and sent to the chemical laboratory. A complete analysis is made for elements which appear in the specification of the steel on order. This analysis is reported to the furnace operator, where it is used as a basis for calculating the ferro-alloys additions which adjust the alloy contents to the desired specification. The ferro-alloys additions are allowed to melt and diffuse uniformally throughout the bath after which another sample is sent to the laboratory. If the analysis of this test indicates that the various elements are present in the proper proportions, the heat is tapped and cast into ingots.

The importance of speed, by which the chemical elements are analyzed and reported, is obvious since the furnace operators cannot proceed with the processing until the exact analysis of the bath is known. The molten steel is maintained under a fused blanket of slag, which may be oxidizing or reducing in nature, and may

vary the percentage of certain elements. Therefore, to be of value, the chemical test must be rapidly analyzed, so that the analysis represents the conditions in the bath before any changes can take place.

Until comparatively recently this chemical control had been accomplished by wet chemical methods. While the accuracy of wet analysis is not questioned, the speed is usually much too slow for present-day production. Ordinary elements such as Mn, Cr, Ni, Mo, Cu and Va may be analyzed within a half hour. Other elements like Si. W, Al, Zr, Ti, B and Co may require as long as 8 to 12 hours to determine the amount present. Often a series of heats is scheduled in which the analysis of the first heat is used as a basis for producing the other heats, hence a prolonged analysis delays production until it can be finished. In the usual steel mill control laboratory one analyst is required, for each element, so that time delays may be held to a minimum. It is evident that quite a staff of chemists is necessary if very complex analysis is required. A laboratory of this type required much valuable floor space with quite a sum spent for chemicals, glassware and apparatus.

Therefore, the need for a more rapid chemical report made the emission-type spectrograph a boon to laboratories. While the spectrograph had been used for quite some time in other fields, the first instrument to be used successfully for ferrous melting control was installed in a foundry in 1935. Since that date many have appeared in the control laboratories of hot-metal industries.

A Baird Spectrograph was installed in the Timken Laboratory in 1943 for the analysis of tests from the melt shop. This instrument is equipped with a three meter focal length Wood's grating, ruled 15,000 lines per inch, set in an Eagle mounting. Samples for spectrographic analysis are cast in the form of rods, $\frac{7}{32}$ inches in diameter, by pouring the molten metal into a mold or sucking it up in a glass tube. The cast pins are sent to the laboratory by a pneumatic tube system, where they are carefully ground to a point of 140° included angle and placed in the electrode holders.

Power from a high voltage arc or spark source is applied and spectral lines from the emitted light are photographed on Eastman spectrum analysis No. 1 plates. The plate is then developed, washed, dried and cooled to room temperature in a dark room. The density of the line for the element required is read on a densitometer and calculated to percent, after which it is reported by telautograph to the furnace operator.

The headaches caused by interfering elements in wet chemistry are eliminated. Spectrographic analysis avoids the necessity of waiting several hours for certain elements, as when wet chemical methods are employed. It is reported that a large steel producer increased tonnage approximately 30 percent after a spectrograph was installed, since heats could be diverted to other specifications prior to tapping, when detrimental elements were present.

With the spectrograph there is a substantial saving in time over the wet chemical methods for special elements such as aluminum, but these elements are not usually requested for control analysis. Control elements are normally Mn, Cr, Ni, Mo, V and Cu. Depending on the number of elements requested, a report of spectrographic analysis can be completed in from 10 to 25 minutes, which time is not too much better than a well organized wet chemical laboratory. Approximately the same number of employees are necessary, if delays are to be held to a minimum.

The last word in rapid control analysis was realized with the development of the direct-reading spectrometer. In this instrument, the photographic stage has been entirely replaced by sensitive electron multiplier tubes, which pick up the emitted light at the various wavelengths and report it as percentage on calibrated clock dials within 35 seconds after a sample has been placed in the electrode holders.

The use of photo tubes eliminates all of the possible errors associated with plates or film, dark room developing and processing, densitometer readings and calculating boards.

A Baird Associates-Dow Direct-Reading Spectrometer was installed in the Timken control laboratory in May 1947 and was the first instrument of this type to be used in the steel industry. A simultaneous analysis of alloys is made; therefore, a total of eight chemical elements can be reported to the melt shop approximately six minutes after a sample has been delivered to the laboratory.

For over three years the direct reader has been used to furnish analyses of all types of steel, except the stainless grades, for elements in the following ranges:

Element	Calibration Range	Element	Calibration Range
Manganese	0.05–1.60	Copper	0.02 –1.20
Silicon	0.01–1.00	Vanadium	0.01 –0.30
Chrome	0.03–7.00	Tungsten	0.05 –5.40
Nickel	0.02–5.00	Aluminum	0.002–1.60
Molybdenum	0.01–1.00	Tin	0.007–0.10

The direct reader is operated 24 hours a day, seven days a week, with one operator on each eight-hour shift, instead of three, as with the spectrograph. The operator can very easily take care of the necessary elements, on all tests, from seven electric and three openhearth furnaces. Operators were selected from the chemical laboratory force, with the thought in mind that, since the number of chemists would be reduced when the spectrometer was installed, it would be essential that these operators could return to the chemical laboratory and carry on control analysis in case of a major spectrometer breakdown. To date this has not been necessary.

The spectrometer is located in an air-conditioned room, which is maintained at a temperature of 75° F and 45 percent relative humidity. It is felt that air conditioning is quite essential to good operation; that is, the atmosphere surrounding the analytical gap is held uniform, but more important, the electronic circuits are not subjected to absorption of moisture due to varying atmospheric conditions.

The instrument is well insulated against shock, the customary vibration found in steel and rolling mills causing no interference. The exit slits, which pass the emitted light from the grating to the photomultiplier tubes, are of the fixed type. In over three years they have never been changed or adjusted, due to varying mechanical or physical changes in the spectrometer. In a recent check, it was found that the light from the spectral lines was still passing through the exact center of each exit slit, which is thought to be quite remarkable, since the light is reflected approximately 24 ft. and the slightest movement will cause the light to miss these narrow slits which are from 50 to 200 microns in width.

Sixty-five electronic tubes are used in the various circuits in the spectrometer, and to date there has never been a shutdown caused by a burned-out tube. During vacation period shutdowns, the tubes are checked and weak tubes are replaced, even though they cause no trouble in operation. Many of the original tubes are still in use, which indicates well-designed circuits.

The location of trouble has been simplified since a channel has been designed for each element and the circuits which control that channel are built into a drawer, which is easily replaced with a spare drawer in case of trouble. The troublesome drawer is then repaired by the operator and held as a spare for future use. Tube fatigue is practically unknown, as shown by experiments in which from 20 to 40 exposures are made in rapid succession with no abnormal variations.

In order to check the effect of a shutdown, the spectrometer was standardized before it was turned off for a three-week vacation period. At the conclusion of this time the power was turned on and the electronic circuits allowed to warm up for 20 minutes. The same standard pins were then sparked and the analyses for all elements were in excellent agreement with the results obtained before the shutdown.

In a recent survey of a two-year period, it was found that results from the

spectrometer were used to control the melting of 14,184 heats of steel. This constituted a total of 951,207 tons, on which 75,470 tests were submitted, making a grand total of 1,021,250 determinations, reported by the aid of the direct-reader.

The real value of savings, possible through rapid control analysis, is apparent since melt shop time can be calculated at approximately one dollar per minute. It is obvious that any gain in analytical time will effect quite a saving, especially when considered on an annual basis. Costly erosion of ceramic furnace linings may be reduced, for with rapid analysis the bath no longer has to be held at approximately 3000° F. while waiting for preliminary analysis before adjusting the alloy content.

It is felt that in the near future this type of instrument will be used quite extensively. With the improvement in accuracy and the shorter period required to report analysis, it is felt that the direct-reading spectrometer is, by far, the greatest contribution that the control laboratory has ever made towards effecting a savings in the melting department. This is especially true today with the emphasis being placed on steel production due to the present emergency.

ABSORPTION SPECTROPHOTOMETRY

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Absorption spectrophotometry in many ways offers a means of analysis which supplements the information obtainable by emission spectroscopy and allows investigation of whole new classes of materials. Consequently, emission and absorption spectroscopy are closely related and instrumentation for both is quite often found in the same laboratory. Before discussing the specific applications of absorption spectrophotometry one should first know of the origin of absorption spectra, become familiar with the general components of the instruments used for measuring absorption spectra, and understand the laws governing the attenuation of radiant energy passing through a homogeneous, isotropic, non-metallic medium.

ORIGIN OF ABSORPTION SPECTRA

Emission spectra consist of sharp discrete lines arising from the transition of electrons from a term level of higher energy to a term level of lower energy in an atom or ion. These term levels can be defined by a principle quantum number, n; an azimuthal quantum number, l; a spin quantum number, s; and the resultant angular momentum, j. The term levels of molecules are designated by a similar system of symbols where Λ is analogous to the azimuthal quantum number, Σ the spin quantum number, and Ω is similar to the resultant angular momentum. In emission spectroscopy the electrons are normally "excited" to the higher energy levels by energy supplied by means of electrical discharge. The amount of energy absorbed in this process is small and is not normally measured, since it is the energy emitted by the excited atoms which serves to qualitatively identify the atoms and to serve for their quantitative estimation.

Absorption spectroscopy is a means of investigating samples by the energy which is absorbed in raising its molecules to excited states. While the phenomenon

[1]Operated by the Monsanto Chemical Company, under AEC Contract Number AT-33-1-GEN-53.

of energy absorption is observed for atoms and ions, this method of analysis is more generally applied to the determination of molecules or particular groups of atoms within a molecule. Since it is made up of several atoms, the energy of a molecule is the sum of the translational energy of the entire system, the vibrational energy, the rotational energy, and the electronic energy of its components. Consequently, it is found that the energy terms for molecules are more complicated than for atoms, and each spectral line of the atomic emission spectrum is replaced by a system of bands. Energy transitions which involve change of rotational energy alone involve relatively small amounts of energy and correspond to bands in the extreme infrared or microwave regions. The vibrational energies are larger so that changes in vibrational (and rotational) energy lead to bands in the near infrared. Transitions involving electronic rearrangement in molecules may lead to absorption of energy in any region of the spectrum. The systems normally used for analytical spectrophotometry absorb energy between 2,000A and 10,000A. In general, the region of the spectrum in which an absorption band occurs is determined by the electronic transition, the gross structure of the system depends on vibrational energy changes, and the fine-line structure of the individual bands depends on changes in rotational energy.

INSTRUMENTATION

Since qualitative spectrophotometry involves measurement of the energy absorbed by a sample as a function of the wavelength and quantitative determinations depend upon the measurement of the absorbed energy at a fixed wavelength, a spectrophotometer must consist of three essential components. These parts are a source of radiant energy, a means of dispersing the energy according to its frequency, and a mechanism for detecting and estimating the amount of energy passing through the sample.

In the ultraviolet and visible portions of the electromagnetic spectrum, three types of sources of radiation are normally used. In the ultraviolet region, from 200 millimicrons to 400 millimicrons, the continuum produced by a hydrogen discharge has been widely used (Lawrence and Edlefson, 1930; Kistiakowsky, 1931). This source gives a continuous source of radiation below about 350 millimicrons and is readily adaptable for use with instruments that contain phototubes or photomultiplier tubes as radiation detectors. Such sources are operated at hydrogen pressures ranging from 1 to 10 millimeters of mercury, at applied voltages from 3,000 to 5,000 volts, and at a fraction of an ampere to several amperes. A hot-cathode, hydrogen-discharge tube has been described (Allen and Franklin, 1939, 1941) which operates at 80 volts and about 1.3 amperes. A ribbon-filament incandescent lamp operating at a color temperature of 2,400° to 2,800° K gives a continuous source of radiation which is usable down to about 320 millimicrons. This limit is imposed by the glass envelope surrounding the filament. A condensed spark between tungsten-steel electrodes is the most widely used source in photographic absorption spectrophotometry. With these electrodes the high intensity of the ultraviolet emission, the richness in lines and their fairly even distribution over the spectral range, the freedom from wandering, and the localization of the luminiferous area in a small space make this source preferred for work in the ultraviolet region. The discontinuous character of the spectrum and the wide range of intensities between the strong and weak lines are disadvantages of this source. These two properties make the spectral image unsuited to the detection of fine structure in absorption bands.

Spectral dispersion and isolation in the region between 200 and 1,000 millimicrons may be obtained by means of refraction through a prism; by the diffraction and interference associated with a fine-line grating; or the spectral isolation may be effected by means of optical filters, there being no dispersing system in the ordinary sense.

Glass or quartz prisms of 60° refracting angle, or equivalent prisms, have been widely used in prism spectrophotometers for the ultraviolet and visible regions. Recently a spectrophotometer has become available which uses a Féry prism. Some instruments have been designed with a double-prism monochromator to reduce stray light.

Gratings have been used to obtain dispersion of the radiant energy, with both transmission and reflection types being of value. A transmission-grating, double-monochromator spectrophotometer is claimed to have an average stray radiation of less than one percent. However, in general, a grating monochromator has a higher relative intensity of stray radiation than a prism dispersing system (Cary and Beckman, 1941).

In certain kinds of work where it has been found adequate to isolate only certain spectral regions, optical filters have proven to be satisfactory. These filters are of three general types: (1) glasses, usually two or more in combination, (2) two or more dyes incorporated into gelatin or other medium, and (3) interference filters. Information on the characteristics of these filtering media may be obtained from the Corning Glass Works (Glass Color Filters, 1946), the Eastman Kodak Company supplying the Wratten dyed-gelatin filters (Wratten Light Filters, 1938), and the Farrand Optical Company (Farrand Interference Filters, 1947) and Baird Associates (Baird Associates Interference Filters, 1946) supplying interference filters.

Spectrophotometry inherently consists of measuring the ratio of two radiant energies at a specified frequency or wavelength, and then repeating this measurement at other frequencies or wavelengths as often as desired over the spectral range of interest. This ratio of radiant energies may be determined by visual, photographic, or photoelectric means. In visual spectrophotometry, the photometric part of the instrument includes a two-part photometric field and a means of varying the luminance of one of the parts so that the eye is used only to detect unmatch and finally to judge match of the two parts of the field. The means of varying the luminance of one part of the field is calibrated, so that the actual value of the match point is determined by this auxiliary system. Photographic photometry is normally used when a conventional spectrograph is used as a dispersing medium. Evaluation of the spectra requires a previous calibration of the photographic emulsion, much in the manner required for quantitative emission spectroscopy. However, because of the higher dispersion normally associated with spectrographs, more fine structure may be investigated by this technique than can be observed visually or with photoelectric photometers. Photoelectric detectors are of three general types: (1) barrier layer cells, (2) phototubes, and (3) photomultiplier tubes. A barrier layer cell consists essentially of a plate of either copper or iron upon which a semiconducting layer of cuprous oxide or selenium has been grown. The semiconducting layer is covered with a light-transparent layer of gold, platinum, copper, or lead which acts as an electron collector. The average cell has an output of about 120 microamperes per lumen and can be used in conjunction with micro- or milli-ammeters to detect and measure high levels of illumination. No external source of potential is required with a barrier layer cell. Vacuum phototubes require a source of potential and are normally used with a vacuum tube amplifier. Over the range of intensities encountered in spectrophotometry, they give a linear response. A special kind of phototube employing several electrodes or dynodes which achieves the equivalent of amplification within the tube itself is called a photomultiplier tube. These tubes have a higher signal-to-noise ratio than the phototubes-amplifier combination.

SPECTROPHOTOMETRIC IDENTIFICATION

Qualitative absorption spectrophotometry may be achieved by comparing the absorption curve of the unknown with the curves of known compounds.

Accurate data on the absorption of a sample as a function of wavelength must be obtained over a considerable portion of the spectrum to allow this method to be used. Similar structures within different molecules will show similar absorptions, and, likewise, similar complex organic molecules will show very similar absorption spectra. In fact, this generalization has been so well established the tables of the wavelengths of the absorptions of different groups in the ultraviolet and visible regions have been made (Harrison, Lord, and Loofbourow, 1948). This general technique has been used to identify the fragments obtained by treating natural vitamin B_1 with sodium sulfite, thus facilitating the synthesis of thiamine (Williams and Ruehle, 1937). This is an empirical method and depends upon having a library of absorption curves or a clue to the composition of the sample so that similar compounds may be prepared.

Unfortunately no single comprehensive compilation of absorption spectra exists. Some data have been compiled by the American Petroleum Institute Research Project 44 at the National Bureau of Standards and is available as the Catalog of Ultraviolet Spectrograms. International Critical Tables contain both absorption data and references to literature containing absorption spectra data on both organic and inorganic compounds. "Tables annuelles de constantes et données numeriques, données numeriques de spectroscopie," Vols. I to XII by V. Henri and L. Bruninghaus contain tabulations of absorption data. Landolt-Bornstein, "Physikalische-Chemische Tabellen," edited by W. A. Roth and K. Scheel; Beilstein, "Handbuch der Organischen Chemie," 4th edition; and Gmelin, "Handbuch der Inorganischen Chemie," 8th edition all contain literature references to absorption spectra. Brode (1945) has made a critical evaluation of most of the available collections of data.

QUANTITATIVE SPECTROPHOTOMETRY

For quantitative determinations a more precise knowledge of the influence of different variables upon the absorption of energy is required. The simplest case of absorption is that in which a parallel beam of monochromatic radiation passes rectilinearly through a homogeneous absorbing medium. Under these conditions the intensity of the radiation is reduced by the same fractional part by each succeeding portion of its path. Thus, if the intensity is reduced by one third in the first millimeter, it will be reduced to one ninth of its original intensity by the second millimeter. This fact was first enunciated by Bouguer in 1729. It was later applied by Lambert and is sometimes known as Lambert's Law. When dealing with solutions, in which molecules are acting as energy absorbers, the optical path may be varied either by changing the cell thickness (Lambert's Law) or by changing concentration of the solute. The latter method, Beer's Law, assumes that the specific absorption per molecule of the absorbing material is independent of its concentration in the solution. These laws are combined in the familiar expression $E = kcd$ where k is the specific absorption coefficient, c is the concentration, and d is the sample thickness. As observed in the use of this technique for qualitative analysis, the extinction of a sample is a function of the wavelength, so quantitative absorption spectrophotometry consists of measuring the absorbancy of sample at a wavelength, or a narrow range of wavelengths, and correlating the extinction with concentration. Quantitative determinations involve merely the solving of simple algebraic equations if the absorbancies of the components of a solution are additive. However, if absorbancies are not additive, quantitative determinations become empirical and depend upon the previous determination of a working curve showing the absorbancy-concentration relationship.

While many instances are known where Beer's Law is said to be valid, deviations are both frequent and significant. Kortum (1936) has stated that "the validity of Beer's Law for ions must be considered as an exception rather than as a rule. Validity over large concentration ranges is not to be expected since Beer's Law is a limit law for low concentrations." The deviations from Beer's Law are of two types—chemical and physical or instrumental.

It is generally concluded that the observed deviations are more a result of chemical changes affecting or occurring in the absorbing ions than in a failure of the law (von Halban and Ebert, 1924). Körtum and Seiler (1939) have summarized the causes for divergence from the law. According to the theory of light dispersion, it is not the specific absorption coefficient k but the quantity $kn/(n^2+2)^2$ which is a constant, independent of concentration. For concentrations lower than 0.01 molar the change in refractive index, n, would rarely cause an error greater than the accuracy of the measurement. Many organic materials have weak acidic properties, and their equilibria are easily disturbed by changes in acidity. For this reason it is often necessary to control the pH of the solution within narrow limits to obtain reproducible results. Equilibria may also be displaced by the presence of non-absorbing materials in the sample through the mass action law. Temperature fluctuations may influence absorption data in two different manners. It may cause a shift in the equilibrium constant of the absorbing system, and it may cause concentration changes due to volume changes caused by expansion. The magnitude of the effect of temperature change depends upon the material. It amounts to 0.1 percent per degree at 436 millimicrons for chromate ion and 1 percent per degree for 2, 4-dinitrophenolate ion (Körtum and von Halban, 1934).

What may be attributed to a deviation from Beer's Law may be a low degree of monochromaticity of the radiant energy used. Even the best monochromators give a spectral band of finite width, and apparent deviations will be noted if the absorbancy of the sample changes considerably across the spectral interval isolated by the monochromator (Brattain, Rasmussen, and Cravath, 1943). Poor matching of absorption cells and stray radiation may also be factors.

TREATMENT OF DATA

Qualitative data are generally presented as curves showing the absorptivity as a function of the wavelength through the spectral range covered. No single method of presentation of this data has become universally accepted. In fact, Mellon (1949) has said "the confusion in usage of terms, symbols, and data in absorptiometry remains unsurpassed by that in any other division of quantitative analysis." The ordinate is usually plotted in increasing values of transmittance, T_i, absorbancy, A_s, (also known as optical density, D, or extinction, E), extinction coefficient, k, (or absorbancy index, a_s), molecular extinction, ϵ, (or molar absorbancy index, a_m) or the logarithm of any of them. The abscissa is used to present the region of spectrum investigated and may be expressed in one or more of the following terms: wavelength, λ, (in millimicrons $m\mu$, or Angstroms, A); frequency, ν (in fresnels); wavenumber, ν' (in waves per centimeter); or the logarithm of the wavelength. Wavelength, wavenumber, and frequency are related by the expression

$$\frac{1}{\text{Wavelength}} = \text{Wavenumber} = \frac{\text{Frequency}}{\text{Speed of Light}}$$

With all possible variations in use, including inconsistency in direction of plotting, and disregard for general good practice in graphing, the literature is likely to be less than clear to anyone unskilled in mentally transforming the curve of unfamiliar form into the form with which he ordinarily deals. Transmittance is often obtained directly from the instruments and therefore used because of convenience. Plotting on a logarithmic scale magnifies the low transmittance values relative to the higher values. Optical density, extinction, or absorbancy magnify the absorption maxima and also facilitate calculation of thicknesses, since the relationship is linear if the solution conforms to Beer's Law.

Quantitative absorption spectrophotometry depends upon the correlation of the amount of energy absorbed at a given frequency with the amount of material

present in the sample. This correlation is known as a calibration or a working curve and consists of plotting transmittance, absorbance, or their logarithms as a function of concentration. Since absorbancy is a linear function of concentration for solutions conforming to Beer's Law, a straight line indicates such conformity. Ringbom (1939) has suggested plotting working curves as absorbancy vs. the logarithm of concentration to emphasize the importance of making measurements within certain transmittancy ranges where the error in measurement is smallest. Ayres (1939) has recently reviewed this technique and pointed out that the minimum error in absorbancy determination occurs at about 28 percent transmission.

USES OF ABSORPTION SPECTROPHOTOMETRY

The chief justification for the development of equipment or methods is the possibility of using them for the determination of the constituents of a sample, or the measurement of properties of systems. Since anions, cations, inorganic groups, organic radicals, and whole molecules all produce systems which are capable of causing the absorption of energy in the ultraviolet and visual regions, the uses of absorption spectrophotometry are so numerous that only the general aspects of the methods and the general fields of applications can be discussed here. For a more detailed discussion of individual applications to analytical problems, some of the standard texts on absorption spectrophotometry should be consulted (Brode, 1945; Snell and Snell, 1948; Welcher, 1947; Mellon, 1950). Reviews of current progress in this field have been published annually for the last three years in the January issues of Analytical Chemistry and present bibliographies to most of the work published in American journals during the year.

Absorption spectroscopy has been applied to problems of determining the isomeric composition of a sample whose constituents are otherwise chemically identical. The analysis of xylene isomers has been discussed by Shostenko and Shtandel (1948). Stair (1949) has discussed the analytical value of the absorption bands in the absorption spectra of four butylbenzenes and three diethylbenzenes.

Complexes which are formed in solution between metals and organic reagents, two organic materials, or two inorganic radicals may be studied by means of absorption spectrophotometry. This method has been used by Molland (1940) to identify the iron complex of 8-hydroxyquinoline-5-sulfonic acid as $(C_9H_6O_4NS)_3Fe$. Evidence for the complex ion $Fe(CNS)^{++}$ was obtained by photometric means (Bent and French, 1941) and later substantiated by migration experiments.

Because of the speed with which measurements can be taken, and more recently because of the development of automatic recording instruments, spectrophotometric analysis is adaptable to the investigation of unstable systems. Probably one of the most fugitive color reactions used for identification is the antimony trichloride—Vitamin A complex where the time of reaction and measurement must be carefully controlled. Readings are normally taken from 5 to 30 seconds after adding the reagent, procedures varying among laboratories. A method for the determination of ascorbic acid based on the decolorization of 2, 6-dichlorophenolindophenol (Hochberg, Melnick, and Oser, 1943) requires that readings be taken at 5 and 10 seconds while the reduction of the dye is proceeding. Gibson and Taylor (1945) have devised a method by which the absorption spectrum can be observed as the sample material flows through the absorption cell. By adjusting the rate of flow one can investigate a system at any predetermined time after mixing the components.

CONCLUSIONS

Given a suitable reaction, a spectrophotometric method can be developed if the intensity of the coloration produced is essentially dependent upon the concentration of the desired constituent, and is stable long enough to permit measurement. The development of specific organic reagents and effective means of complexation

have made possible the determination of single components found in complex systems. Spectrophotometric methods, not being limited to elements, can often be applied to the determination of compounds, radicals, and functional groups. Furthermore, chemical phenomena which occur in solution, as complex formation, solvation, and equilibria, may be studied in situ by spectrophotometric methods. Absorption spectrophotometry thus provides a useful analytical and research tool which bridges the span between the data on atomic and ionic species supplied by emission spectroscopy and the information on molecular and crystalline states obtained from X-ray diffraction.

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ANALYSIS USING INFRARED SPECTROSCOPIC METHODS

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REQUIREMENTS OF A PHYSICAL-ANALYTICAL METHOD

The essential requirements in any analysis based on a physical method is that there must be some physical property of the substance being analyzed that is unique. Thus, for example, if one has a mixture of two substances, the density of each of which is known, the composition of the mixture can be determined by measuring its density. Or, the physical property may be the index of refraction. If the indices of refraction of the components of a mixture and the index of refraction of the mixture are known, it may be possible to determine the composition of the mixture. In this case, mixtures of more than two components may be analyzed if the indices are known for more than one wavelength. These physical properties are satisfactory for an analysis of a mixture of substances whose identities are

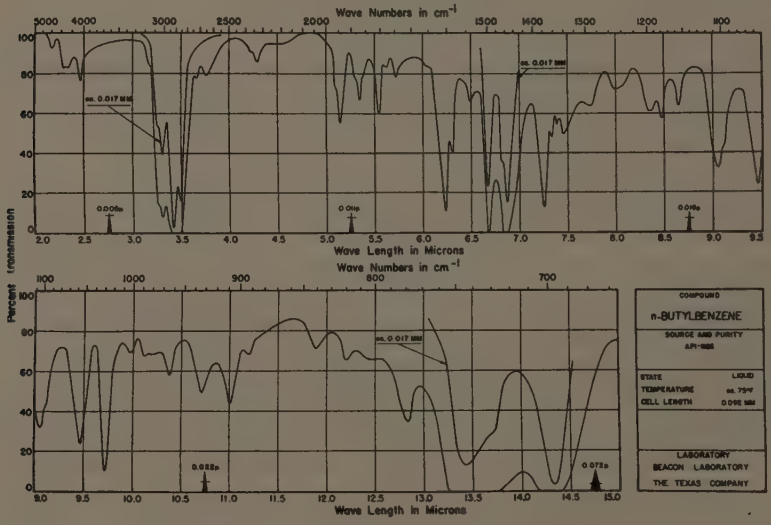

FIGURE 1. Infrared Spectrogram of n-butylbenzene. (Courtesy The Texas Company.)

known. If many components are involved or if the exact nature of the components is not known, the analysis becomes more complicated. One must depend either upon the determination of several physical properties or upon a physical property which has several values associated with it for each constituent of the sample being analyzed. Because ultraviolet, visible, infrared, or x-ray data or the mass spectrum have many numbers associated with a given substance, these are properties which may permit analysis of a complex mixture by using only one such property. The use of two or more such methods will extend the field of usefulness still further. Although the following discussion refers directly to the use of the infrared spectrum as a means of analysis, much of it would be equally applicable to other methods.

Figure 1 is a spectrogram[1] of a typical hydrocarbon, n-butylbenzene, in which it may be seen that in the wavelength interval between 2 microns, i.e., 20,000 Angstroms, and 15 microns, there is a large number of absorptions. (Absorptions are represented by valleys in the curves). A spectrogram for any other compound, even one so similar as iso-butylbenzene, would be quite different, in that there would be absorptions at different places in the spectrum. Some of the absorptions occurring at the same wavelengths would have different intensities in the two spectrograms. This illustrates that infrared spectroscopic data satisfy the requirement that many values characterize the substance in question. Thus the frequent reference to infrared spectra as fingerprints of molecules is justified.

ORIGIN OF INFRARED SPECTRA

What is it about the molecules that gives rise to the different infrared spectra? Basically, these spectra result from the fact that the molecules are vibrating and rotating. A molecule is characterized by many energy levels. Figure 2 is an oversimplified diagram of energy levels for a hypothetical molecule. The longest

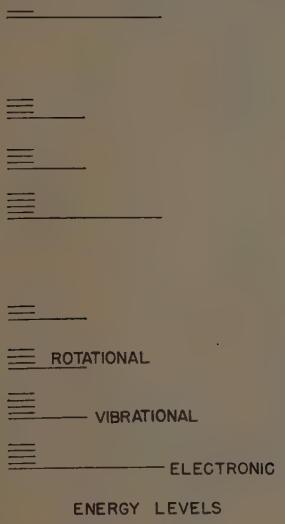

FIGURE 2. Simplified energy level diagram for a hypothetical molecule. Longest, intermediate and shortest lines correspond respectively to energy levels for different electronic, vibrational and rotational states.

lines, representing electronic energy levels, are farthest apart; vibrational energy levels are closer together; and rotational energy levels are separated by the smallest interval of all of these. If electromagnetic energy falls upon a molecule under proper conditions, it may absorb some of that energy and be raised to a higher energy level. Thus for example if a photon having the proper amount of energy hits the molecule, the molecule may change electronic states. Or if the energy is somewhat less, it may change vibrational states. If a still smaller amount of energy characterizes the photon, it may give rise to a change in rotational levels. Frequently the absorption of energy may result in changes of states of all three types. The energy ΔE, transferred in such a change is related to the frequency ν through the relation:

$$\Delta E = h \nu \ (\text{sec}^{-1})$$

[1]Spectrograms of this type are to be found in the Catalog of Infrared Spectrograms compiled by the American Petroleum Institute Research Project 44 at the Carnegie Institute of Technology under the direction of Dr. F. D. Rossini.

where h is the Planck constant. The frequency is related to the wavelength λ by

$$\nu\ (\text{sec}^{-1}) = \frac{c}{\lambda\ (\text{cm})}$$

where c is the velocity of light expressed in cm/sec. The reciprocal of the wavelength, i.e., the number of waves per cm, is commonly referred to as the "wave number" and is designated by ν'. Then:

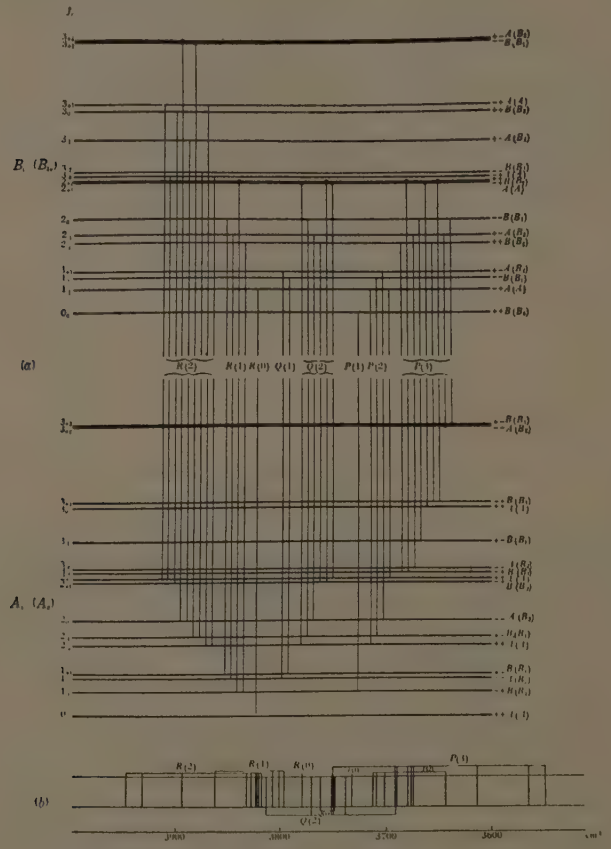

FIGURE 3. Energy level diagram for one band of water vapor. (Courtesy G. Herzberg and D. Van Nostrand Co., Inc.)

$$\frac{\nu\ (\text{sec}^{-1})}{c} = \frac{1}{\lambda} = \nu'\ (\text{cm}^{-1})$$

and

$$\Delta E = \frac{h\,c}{\lambda\ (\text{cm})} = h\,c\,\nu'\ (\text{cm}^{-1})$$

Thus the change in energy is inversely proportional to the wavelength or directly proportional to the frequency or wave number. The chief thing to note is that since the electronic transitions involve large amounts of energy, the frequency of the radiation is large and the wavelength is short. Thus this type of transition leads to absorptions in the visible or ultraviolet portion of the spectrum. Changes in vibrational levels involve somewhat less energy and give rise to absorptions in the infrared region in which we are interested. Changes in rotational level involve still longer wavelengths and are associated with absorptions in what is known as the far infrared spectral region, i.e., radiation whose wavelengths are 25 microns or longer.

The presence of different atoms in a molecule, or a different spatial arrangement of the same atoms results in a different vibrating-rotating system. All such systems represent problems which may be solved by the methods of mechanics. Actually these problems are so complicated that theoretical physicists have largely confined their efforts to attempts to relate the wavelength of absorption with the frequency and the normal mode of vibration of only the simplest molecules (Wu, 1939; Herzberg, 1945, 1950). Fortunately for the analyst, it is no more difficult to obtain experimental data from a complex molecule than from a simple diatomic

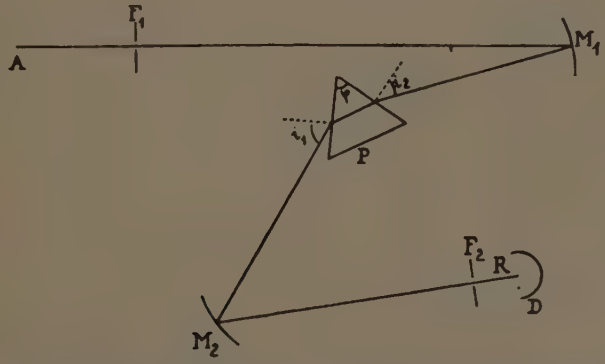

FIGURE 4. Simplified diagram of a spectrometer. A is the source, F_1 is the entrance slit, M_1 is a collimating mirror; P is a dispersing device (a prism here). M_2 is a focussing mirror. F_2 is an exit slit. R and D are the components of the radiation detector.

molecule. If a photon approaches a molecule and if the energy of the photon is equal to the difference in energy states of the molecule, there is a finite probability that that molecule will absorb that photon. Having many molecules in a beam of light, some of them are constantly absorbing radiations of all wavelengths corresponding to energy level separations for which transitions are permitted. Figure 3 is a diagram showing a few of the energy levels and permitted transitions for one of the bands of the water molecule. This illustrates that these transitions are numerous and that the spectra become complicated.

INSTRUMENTATION

The next question of interest is how one ascertains these differences in the spectra of various compounds. Figure 4 is a simplified diagram of a spectrometer used to obtain such spectra. A is a source of radiation; F_1 is an entrance slit; M_1 is a mirror which collimates the radiation. The parallel rays of radiation traverse the dispersing device represented by the prism P. The mirror M_2 focuses

the radiation on the exit slit F_2 after which it reaches the detector R. The source, the prism, and the detector will be considered briefly.

Source. In obtaining infrared absorption spectra, it is most convenient to use an incandescent body which emits radiation of all wavelengths in the regions to be investigated. Most of the sources used have emission characteristics approaching those of a black body. Figure 5 contains the familiar series of curves of black body sources radiating at different temperatures. This illustrates the fact that such sources, although continuous, do not have uniform intensity throughout the spectral region. It is further to be noted that although the curves extend only to about 6 microns at the long wave end, even at this relatively short wavelength

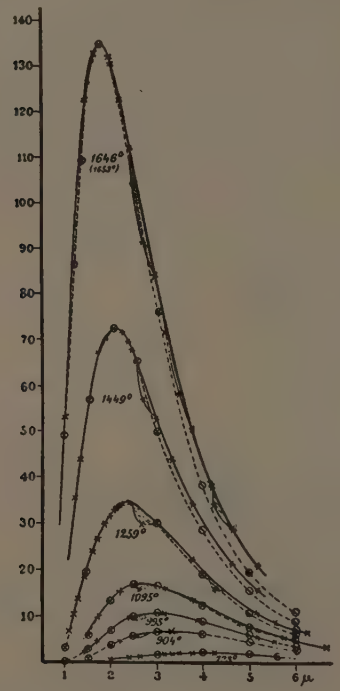

FIGURE 5. A family of black body curves, showing the relative amounts of radiation emitted as a function of wavelength. The number associated with each curve is the absolute temperature.

there is a considerable decrease in intensity. The Rayliegh-Jeans law which applies well at longer wavelengths for sources at these temperatures indicates that the intensity falls off inversely as the fourth power of the wavelength. Hence at 15 or 25 microns, there is relatively little power available. This puts a limitation on the range of wavelengths which may be examined readily. However techniques have been developed so that there is no great difficulty in determining absorption characteristics at wavelengths as long as 25 microns. The Nernst glower and the Globar are the two most commonly used sources of radiation. They both operate in the temperature range of 1000 to 1500 degrees Kelvin.

Dispersing device. The next component considered is the dispersing device. In figure 4, a prism is shown. Gratings are sometimes used for this purpose. The three requirements upon a prism are that it be transparent to radiation of the region being investigated, that it be characterized by relatively high dispersion, and that the material be available in form suitable for making a prism. If the

index of refraction is plotted against wavelength, it is seen that the highest dispersion is obtained in the wavelength region just shorter than that at which the material becomes opaque. Table 1 lists the wavelengths below which materials transmit radiation, hence indicates the region in which that material is the best for making a prism. The only comment that needs to be made with respect to this table is that the material labelled KRS-5 is a mixture of thallium bromide and thallium iodide.

TABLE 1

Wavelengths below which materials transmit radiation

Material	Wavelength
Glass	2.5 microns
Quartz	4.0 "
LiF	5.5 "
CaF_2	12.0 "
NaCl	15.0 "
KBr	25.0 "
KRS-5	45.0 "

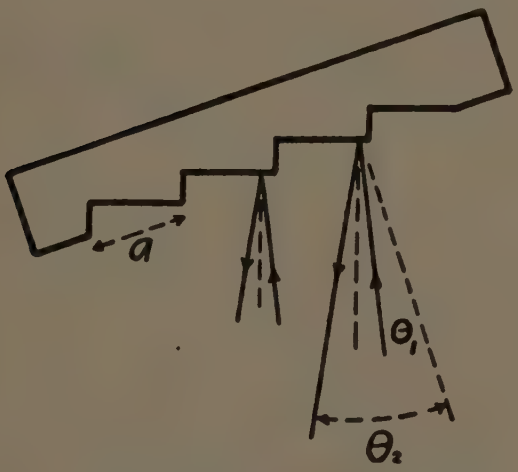

FIGURE 6. Diagram of the face of an echelette grating.

The other type of dispersing device commonly used is a grating. Because relatively coarse gratings are required in infrared spectroscopy, it is common to use an echelette grating (Wood, 1910). The shape of the grooves in an echelette grating is shown in figure 6. The grooves are step-shaped, with the result that the radiation may be concentrated in a given direction. This is important because it means that it is possible to obtain most of the radiation of a given wavelength in one order. It should be emphasized, however, that the radiation of other orders and different wavelengths will be reflected in the same direction in accord with the usual grating formula:

$$n\lambda = d(\sin i + \sin \theta)$$

where n is the order, d is the spacing between the rulings on the grating, i is the angle of incidence and θ is the angle of diffraction. Hence there is still a problem

of filtering that is sometimes a difficult one to solve in grating spectroscopy. Gratings rather than prisms are ordinarily used when the highest dispersion is required. The result is that the presence of spectral lines which are closer together may be observed. To the analyst, this means that a larger number of physical quantities characteristic of that substance is available. Hence there is a greater possibility that this tool may be used as a means of identifying the substance in question. It is true that today most infrared analyses are being conducted on prism spectrographs. This is not to suggest that such will always be the case. Perhaps not many years hence grating spectrometers will be used extensively for analytical purposes. Figure 7 illustrates graphically what may be achieved through

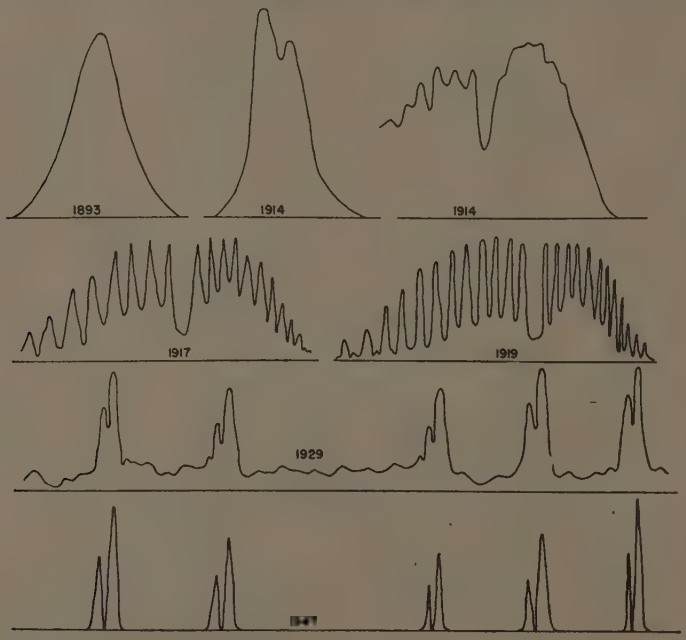

FIGURE 7. The 3.5 micron HCl vapor band examined using the highest resolving power available at the date under each curve. Absorption is plotted upward in these curves.

the use of higher resolution. This happens to be a historical display of the best resolving power available at different dates. A portion of the 3 micron hydrogen chloride band is shown. In 1893 it was known that there was a single absorption in this band, absorption being indicated upward in this figure. By 1947 the band had been separated into many pairs of lines, a few of which are shown at the bottom of the diagram.

Detector. The other component of the instrument of some interest is the detector. Devices used for detection of the presence of infrared radiation may be arranged in three classes. Most widely used is the thermocouple or thermopile. This is not the laboratory type of twisted iron and constantan wires, but is rather a delicate, extremely sensitive device which nevertheless operates on exactly the

same principle as the heavier ones. The second type of detector is a bolometer which is a sensitive resistance thermometer, sometimes put in a modified Wheatstone bridge. A third type of detector is a gas thermometer. Like the other detectors this is of minute dimensions and has several special features associated with it, with the result that it, too, is capable of showing the presence of small amounts of radiation. The minimum power which may be measured by any of these devices ranges from a microwatt downward.

General remarks. So little has been said about the instrumentation as a whole that the impression may be conveyed that it is simple. Such is not the case. The desire to obtain data rapidly and in a most useful form has served as a stimulant for instrument designers to build into spectrographs the ability to subtract the absorption due to solvents and to record data as percent transmission. This is all done automatically and quickly on some spectroscopic instruments available commercially.[2] As a result of these engineering developments, instead of being a very simple optical instrument, the infrared spectrograph has become an electronic instrument with the optical components housed in one of the smaller boxes comprising the instrumentation.

The samples may be examined in a form of a solid, a liquid, or a gas. If it is a solid, such as a thin film of plastic or rubber, it is only necessary to support it in the radiation beam. Otherwise it may be necessary to make a mull or solution or to otherwise prepare it for use. If it is a liquid, the liquid is ordinarily inserted between a pair of transparent plates, such as sodium chloride, potassium bromide, or silver chloride with an appropriate spacer. If the sample is a gas, it is merely necessary to have a tube, closed with appropriate windows on the ends, which will hold enough of the sample to obtain the desired absorption.

QUALITATIVE ANALYSIS

All of the foregoing is introductory to the matter of analysis. Now that the absorption spectrogram can be obtained, what can be done with it? That is, how does one interpret the spectrogram of the unknown substances? The investigator should have some idea of what is contained therein. Even if he does not, the chances are that the spectrogram will give some clues to the experienced observer. For example, absorptions between 3.2 and 3.5 microns suggest the presence of C-H bonds. Absorptions in the 6 micron region may be indicative of double bonds such as C=C or C=O carbonyl groups. Furthermore, the exact wavelength of the absorption will assist in identifying the type of carbonyl group if that is indicated. This is the type of interpretation that is useful in a qualitative analysis. Ultimately the analyst will hope to compare this spectrogram with a spectrogram of a pure substance and find the two to be identical. This means that it is necessary to know the spectrogram of the pure compound if a complete analysis is required. This is no more unreasonable than demanding that the exact index of refraction of a substance be known if one wishes to use the refractive index method for analysis.

QUANTITATIVE ANALYSIS

In undertaking quantitative analyses, there should be available the spectrograms of the pure components as well as that of the mixture whose composition is to be determined. If there exists a particular wavelength at which only one component absorbs, determination of the magnitude of the absorption at that wavelength will be a measure of the concentration of that component. Such ideal situations rarely occur. Ordinarily, all components absorb radiation of all wavelengths to some extent. This necessitates an inquiry into the laws governing the absorption

[2]Literature describing infrared spectrophotometers available commercially may be obtained from: Baird Associates, Cambridge, Mass.; Beckman Instruments, Inc., S. Pasadena, California; and Perkin-Elmer Corporation, Glenbrook, Conn.

of radiation. While this is not so simple a matter as may at first appear, with the result that there are still some ambiguities about these laws, it is frequently the case that the exponential law, variously referred to as the Lambert-Beer-Bougert law is applicable (discussed by Dr. Timma in the preceding article). As a result of the working of this law, the amount of a substance is proportional to the optical density which is defined as the logarithm of the reciprocal of the radiation transmitted by the sample. Furthermore, if there are several substances present, in the simplest cases the optical densities at a given wavelength are additive. The proportionality constants, which are different at each wavelength for each substance, may be, at least in principle, determined from the spectrograms of the pure substance.

In the quantitative analysis of a multi-component mixture, there results a series of simultaneous linear equations. Thus if one is analyzing a mixture for four possible components, it becomes necessary to make the analysis at four different wavelengths. These wavelengths are ordinarily chosen as those at which the absorption of the supposed constituents are most uniquely characteristic of that component. Putting constant obtained from the spectrogram of the known constituents into the equations, one may solve for the concentrations of these four constituents. The solution of four simultaneous equations is not always simple. Furthermore, if one is attempting to obtain solutions to six or eight or ten or twelve simultaneous equations, normal methods of solutions learned in college algebra are not practical. These, however, may be solved by interative methods (Crout, 1941). Or, better yet, there are available electronic computers (Morgan and Crawford, 1944; Berry et al., 1946) which may be used to solve such simultaneous equations by setting up electrical circuits which are analogous to the equations involved.

To summarize, the method of analysis involves comparison, on a qualitative or quantitative basis, of the spectrogram of the sample being analyzed with spectrograms of substances presumed to be constituents of the sample.

EXAMPLES OF ANALYSES

What kind of analyses can be made using infrared spectroscopic methods? Probably the best answer to this can be obtained by referring to articles by Barnes and Gore (1949), and Gore (1950, 1951) which survey the uses of infrared spectroscopy. Each of these articles covers the developments for a one-year period. In each article there are between three and four hundred references to the literature. This shows somewhat of the scope of the application of the method. A few examples will be discussed briefly which will indicate types of analyses that can be made.

An interesting application was made by Coy, Sabo, and Keeler (1949) at the Squibb Laboratories at New Brunswick, New Jersey. Methods were developed for determining the potency of aged penicillin salts. It had been determined that a particular absorption band is present in the penicillin salt but not in any degradation products. Therefore, by making an analysis at one particular wavelength, it was possible to tell how much of the salt was still biologically active. Comparison with biological tests showed excellent agreement.

Another example, not altogether different from the foregoing, relates to the question of whether lubricating oils are from a Pennsylvania crude base. It has been found by Fred and Putscher (1949) that all Pennsylvania crude oils have an absorption band at 10.3 microns. With minor exceptions, others do not. In particular, certain Texas crudes which have color and other physical properties resembling the Pennsylvania crudes have no absorption at this band. Therefore, if one wishes to assure himself that he is obtaining Pennsylvania oil, or that he is not obtaining Pennsylvania oil, depending upon his desires, his car should be equipped with an infrared spectrograph. It is thought that this 10.3 micron

band may be due to an olefin with an internal unsubstituted double bond, since many olefinic compounds with such double bonds also have absorptions there.

Methods have been developed for quantitative analysis of alpha, beta, gamma, delta, and epsilon isomers of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane (Daasch, 1947). The gamma isomer of this particular compound is an excellent insecticide. In order to satisfy governmental regulations pertaining to the purity of this substance, it was necessary to know the exact concentration of the gamma isomer. Techniques were developed for determining the presence of the isomers to less than 0.1 % of the total sample.

A similar problem solved by Anderson and Zerwekh (1949) involved the development of a method for the determination of the amounts of contaminating hydrocarbons in concentrates of n-heptane.

A liquid analysis, not involving concentrates, is described by Perry (1951). In this investigation, mixtures of five C_{10} aromatics were analyzed. Synthetic mixtures showed that the accuracy of the method averaged about plus or minus 0.5% of the total sample. This is typical of liquid analyses using these techniques.

SUMMARY

Molecules that have different structure vibrate and rotate differently, hence have different infrared absorption spectrograms. Apparatus is available for detecting these differences. This satisfies the requirements for a method of chemical analysis based on differences in a physical property of the substances. That the method is satisfactory has been demonstrated in hundreds of applications.

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CHEMICAL ANALYSIS BY X-RAYS[1]

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In the earlier days of the century pioneer work such as that of Moseley (1913), von Hevesy (1932) and von Hevesy and Alexander (1933) opened up tantalizing possibilities for rapid, efficient, and accurate chemical analysis. It is an interesting fact that during the ensuing thirty years of intensive instrumental development these possibilities remained largely beyond the grasp of workers in the physical sciences. Only recently have methods of x-ray production, utilization, and measurement evolved to the degree that some of the promises can be realized in practice. It is the purpose of this paper to review the progress of x-ray analysis, to emphasize its unique advantage in certain cases, and to indicate directions in which developments are proceeding at the present time. There will be no attempt at an exhaustive survey of the field.

Two broad classifications of chemical analysis by x-rays may be distinguished, which we might call ultimate analysis and compound analysis. The former tells us what chemical elements are present in the sample, irrespective of their chemical combination, and, if we wish, the percentage composition. Compound analysis yields information not only on the elementary composition but also gives the particular chemical compound. By this means, for example, we could determine that a certain white crystalline powder is composed of a mixture of NaBr and KCl and not NaCl and KBr. Compound analysis likewise can be made to give quantitative results by use of the proper techniques.

Both ultimate and compound analyses depend for their principles of operation on the diffraction of x-rays by crystals. It will be recalled that a crystal may be thought of as consisting of a multitude of layers or planes of atoms, each plane separated from its neighbor by a grating space, d, characteristic of the crystal. These many layers may cooperate in such a way that the atomic planes appear to an incident x-ray beam very much the same as does an ordinary mirror to a beam of light. If, then, a monochromatic beam of x-rays of wavelength λ is incident on these atomic planes at an angle θ, specular reflection will occur *provided* that in addition the following condition is satisfied, namely,

$$n\lambda = 2d \sin \theta. \qquad (1)$$

In this equation, the so-called Bragg equation, n is an integer, called the *order* of reflection and in practice rarely exceeds 4 or 5. If the incident beam is heterogeneous in wavelength *only those wavelengths satisfying Eqn. 1 will be reflected* for any given angle of incidence. The remainder of the beam will continue on into the crystal and eventually be absorbed. By varying the angle θ, then, one can sweep through the spectrum and determine which x-ray wavelengths are present.

Using these principles of operation W. H. Bragg and his son, W. L. Bragg (1913), constructed the spectrometer shown schematically in figure 1 for the analysis of x-ray emission spectra from various elementary targets. This design principle is of particular interest to us, for it is still in use in the popular and effective North American Philips Geiger Counter Spectrometer, of which we will have more to say presently.

In the Braggs' original spectrometer the x-rays were generated in a gas type x-ray tube, the only kind known at the time. The ionization chamber has such

[1]Acknowledgment is made of support of some of the work mentioned in the article from funds granted by the Ohio State University Research Foundation to the University for aid in fundamental research, by The Research Corporation of New York, and by an Office of Naval Research contract.

an angular position with respect to the crystal planes that any radiation in the incident beam which is reflected from the crystal according to Eqn. 1 will enter the ionization chamber. By a gearing arrangement the ionization chamber is made to move at twice the angular velocity of the crystal table so that this relation is preserved at all Bragg angles.

In their book the Braggs (1925) consider both the case in which the crystal C in figure 1 is a large single crystal and that in which C is a powdered crystal and discuss the difference in the resulting curve of the reflected intensity versus the Bragg angle.

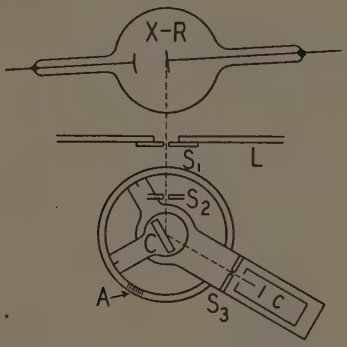

FIGURE 1. Bragg X-Ray Spectrometer. S_1, S_2, S_3, defining slits; L, shielding lead box surrounding the tube; C, analyzing crystal; IC, ionization chamber; A, divided circle.

ULTIMATE ANALYSIS

In their early experiments the Braggs (1913) showed that a typical emission spectrum from a single element has two components (fig. 2). The first, the *continuous* or "white" spectrum, has a diffuse distribution in wavelength, starts abruptly at the short wavelength limit, whose position is determined solely by the target potential applied to the x-ray tube, rises to a broad maximum and trails away at long wavelength. The *characteristic* spectrum, superposed on the continuous, is in sharp contrast, for it consists of narrow, intense "lines," whose wavelengths are characteristic of the target material alone. Each element has its own characteristic spectrum. The lines are separated into groups, usually called *series*, the shortest wavelength series being named the K series, followed by the L, M, N, . . . series.

Figure 2 gives the entire K series of iron. Its utter simplicity as compared with the ordinary arc spectrum in the visible region, containing thousands of lines, is a noteworthy feature. The precise measurement of the wavelength of any one of the x-ray lines provides certain identification of the presence of iron in the target material. The presence of other elements has none but the minutest effect on the wavelength or shape of these lines. This, for the most part, is a separate subject and will not concern us.

The problem of ultimate analysis by emission, stated simply, is: To produce characteristic x-radiation from the sample, to analyze this radiation into a spectrum by crystalline reflection (or other means) and to detect, measure and interpret the spectral distribution in terms of the elementary constitution of the sample and the relative percentages. In principle the solution to this problem has been well known for some years for the great majority of elements from Be(4) to U(92) and higher. As a matter of ordinary laboratory routine the complete solution is still nowhere in sight; but if we will be content with something less than perfection x-ray analysis can be of very considerable value to the routine analyst.

Several treatises (von Hevesy, 1932; von Hevesy and Alexander, 1933; Glocker, 1936; Clark, 1940) and many papers have been written on x-ray analysis. The attention of the reader is called particularly to the lists of advantages and disadvantages of x-ray analysis in Professor Clark's book. It will become clear as this article proceeds that modern techniques have relinquished none of the advantages and have eliminated not a few of the disadvantages. The difficulties inherent in the method have been subjected to close scrutiny and necessary precautions outlined. The application of the method to any particular problem in analysis must be studied by the prospective user if he is to have reasonable certainty of success. Once applied properly, however, the method is simple, foolproof and gives a unique answer. In routine cases anyone of ordinary intelligence can quickly learn to perform the necessary manipulations.

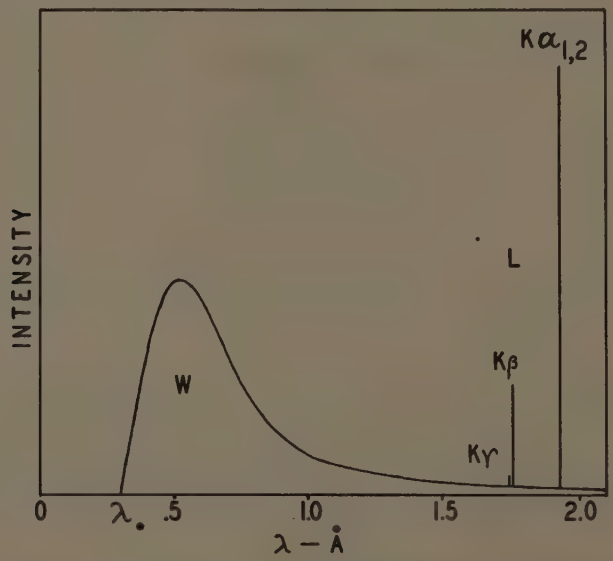

FIGURE 2. Intensity *vs.* wavelength from an iron target showing the continuous spectrum, W, with its short wavelength limit, λ_0; and the K characteristic spectrum L. Relative intensities are only indicative. A target potential of 40 KV is assumed.

Chemical analysis by x-rays as described in the above treatises might be referred to as "classical analysis." Here, for the most part, the photographic plate or film is used for registration of the spectra. Clark (1940) lists four possibilities for x-ray excitation of the sample:

1. The sample is made the target of the x-ray tube and direct electron bombardment is used.

2. Secondary fluorescent emission is excited by placing the sample *inside* the x-ray tube in such a position that it is screened from the cathode rays but subjected to primary x-radiation from the target.

3. Secondary fluorescent emission is excited by placing the sample outside the x-ray tube immediately in front of the window.

4. Cathode rays emerging from the tube through a very thin window produce electron bombardment excitation of the sample outside of the tube.

Of these methods Nos. 1 and 2 will give the greatest intensity of x-rays but require a demountable tube with its attendant pumps and troubles. We must

discard consideration of these methods if we are going to speed up and simplify the process. No. 4 is strictly a laboratory technique and must be disregarded.

We are left with the single choice of the third alternative, with its attendant disadvantage of low radiation intensity. If, in addition, we must depend on photographic registration of the spectra the method becomes too slow and tedious and insensitive to be practical in routine analysis. This was the situation until the Geiger Counter (Korff, 1946) and the Scintillation Detector (Hofstetter and McIntyre, 1950) were developed into dependable measuring instruments during the last ten years.

Both the Geiger Counter and the Scintillation Detector have the unique advantage that they detect and register single x-ray quanta which enter the

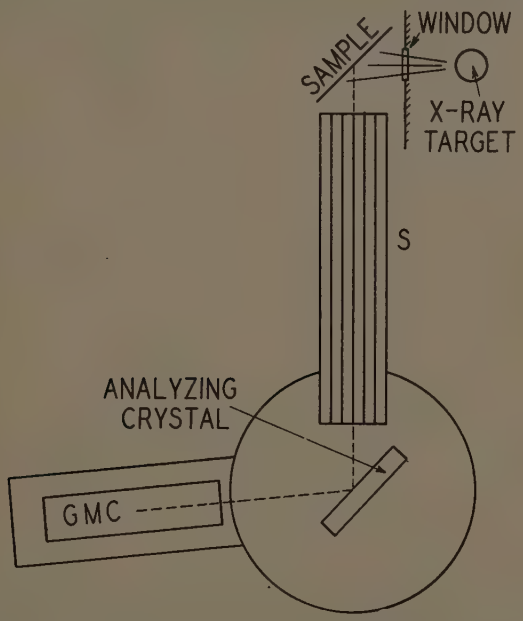

FIGURE 3. Schematic diagram of Friedman's (1948) arrangement for ultimate analysis by fluorescent x-rays. The multiple slit S is described in the text.

detector. In the case of the Geiger Counter the x-ray quantum enters the tube through a transparent window and, upon absorption in the gas contained in the tube, starts an electrical discharge between two electrodes provided for the purpose. Provision is made to extinguish the discharge after ten to a hundred microseconds and the resulting pulse of electrical charge actuates an electronic relay to record the arrival of the x-ray quantum. The x-ray intensity is recorded automatically as the number of quanta entering the Geiger Counter per second of time. Geiger counters suitable for x-ray detection with their attendant electrical circuits are available as standard equipment from manufacturers of x-ray equipment.

The Scintillation Detector is not yet in commercial use on a wide scale. The principle depends upon the well-known fact that when certain crystals absorb an x-ray quantum a flash of light is emitted. This fact is, of course, made use of in the ordinary x-ray fluoroscope used by the medical profession. The Scintillation

Detector uses a photomultiplier tube, such as the RCA 5819, to detect the flash of light and record it as an x-ray quantum absorbed in the crystal. Again, the number of such quanta registered per second is a measure of the x-ray intensity. The Scintillation Detector has several attractive advantages over the Geiger Counter. The quantum is registered in no more than one microsecond, and the system is then prepared to register another quantum. For this reason, then, a considerably higher intensity can be recorded with the Scintillation Detector than with the Geiger Counter. A second point is that no entrance window to the crystal is required. This would be especially desirable for recording very soft x-rays, as we will see presently.

Friedman and Birks (1948) have explored some of the possibilities of the method using the apparatus shown schematically in figure 3. The sample under investigation is placed outside the tube and as close to the window as possible.

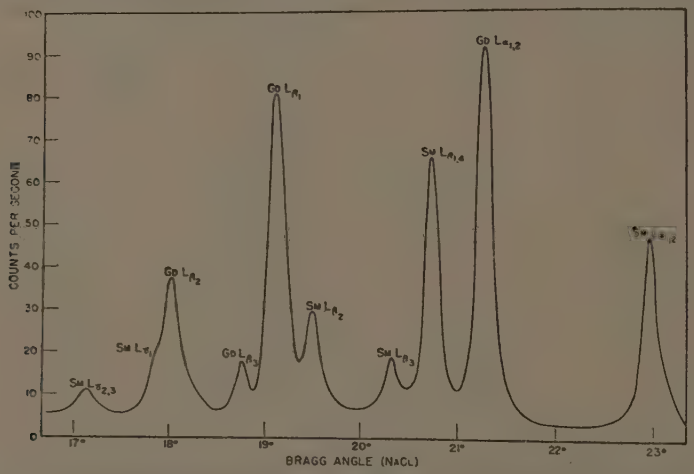

FIGURE 4. Fluorescence spectrum of the L series lines of a mixture of samarium (62) and gadolinium (64) salts (after Friedman, 1948).

The multiple slit S, through which the secondary radiation passes to the analyzing crystal, is made of a bundle of small diameter, thin walled nickel tubing. This produces a satisfactorily parallel beam of fluorescent x-rays. The Geiger Counter is arranged to receive the x-rays reflected at the Bragg angle from the analyzing crystal of rock salt or fluorite. The x-ray intensity is recorded on an automatic chart recorder as the Bragg angle is varied continuously by means of a small synchronous motor which drives the crystal and Geiger Counter arm.

Figure 4 shows the result when the sample consists of a mixture of two rare earth salts—samarium (62) and gadolinium (64). It is immediately apparent that even though the L series contains more lines than the K series there is no ambiguity about the identification of the constituents. The remaining elementary components of the salts above atomic number 22 could likewise be detected by measurement of their characteristic radiations from the sample. The relative heights of the lines depend on several factors and are not usually predictable with accuracy from the percentages of the constituents in the mixture alone.

Figure 5 shows how the measured intensity of K series radiation varies with atomic percentage of iron in alloys of iron with aluminum (13), with nickel (28) and with silver (47). For quantitative analysis, then, one must make up control

samples for calibration purposes, as is necessary with optical spectroscopy. The only particular care required in excitation of the sample is that the primary x-ray intensity must be maintained constant. This is most easily accomplished by electronic regulator systems for the x-ray tube voltage and emission current (Le Mieux and Beeman, 1946; Pepinsky and Jermotz, 1948).

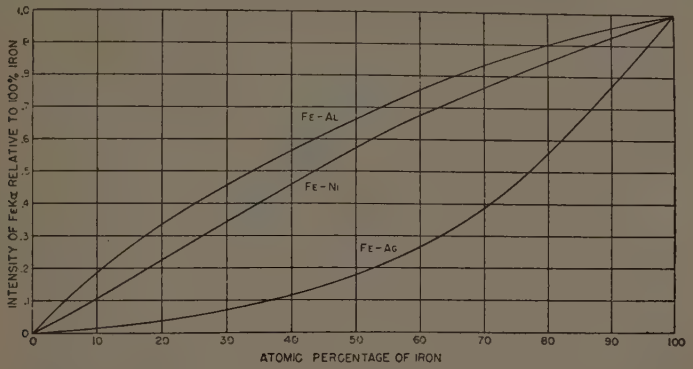

FIGURE 5. Relative intensity of FeKα radiation *versus* atomic percentage of iron in mixtures with aluminum, nickel and silver (after Friedman, 1948).

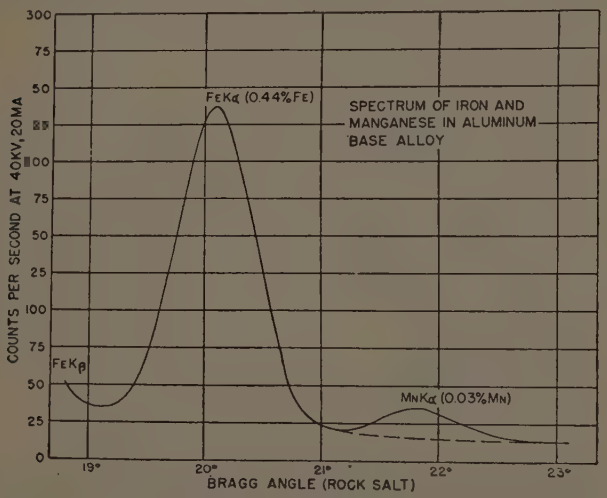

FIGURE 6. Spectrum of iron and manganese in an aluminum base alloy. (After Friedman, 1948).

Figure 6 illustrates the sensitivity of the method. The reader is referred to the original article for details of the investigation. It will be noticed that in this exploratory investigation the apparatus was an assemblage of components not specifically designed for this particular application.

The North American Philips Company has recently placed upon the market a complete fluorescence analysis unit specifically designed for analysis above atomic number 20.

It would be of great advantage to be able to extend the wavelength region. By going to 5.5A, for example, all atomic numbers greater than S(16), including those between Mo(42) and Cs(55), inaccessible to Friedman, would be made available without exceeding 45 KV on the x-ray tube. If the region could be extended to 12A, the practical limit for crystals, the atomic number range would include Na(11). This would necessitate placing sample and spectrometer in a chamber which may be evacuated. Notice that the x-ray tube itself need not be in the chamber.

The main difficulty encountered is that Geiger counter tube windows would have to be designed to withstand both the inward atmospheric pressure and the outward zero pressure of the evacuated chamber and at the same time be so thin that the long wavelength radiation will be satisfactorily transmitted. This seems to be an unreasonable requirement for the Geiger Counter, hence the interest in the scintillation counter, which has no window.

Recently West, Meyerhoff, and Hofstetter (1951) have described experiments on the measurement of x-ray intensities to 6A with single, optically clear crystals of NaI sensitized with thallium. These crystals, suitable for scintillation counters, are obtainable from The Harshaw Chemical Company. They are hygroscopic and extremely poisonous. The hands must be washed immediately after handling this material and care taken that none of the dust is inhaled. They may be stored and cleaved under Nujol. It is entirely possible that the crystals may be satisfactorily coated against atmoshperic moisture by dipping them into a solution of polystyrene in benzene and allowing to dry. It is already known that their performance is not impaired by a coating of Nujol (Hofstetter and McIntyre, 1950).

There seems to be no obvious reason why the atomic number range should not be extended to make ultimate qualitative and quantitative analysis under favorable circumstances (Clark, 1940), a simple, straightforward procedure for the large majority of chemical elements.

ULTIMATE ANALYSIS BY ABSORPTION

In certain cases of quantitative analysis the preparation of a control sample to use with the emission method is either inconvenient or impossible. Many ores would furnish satisfactory examples of the former and that rarest of elements (Dushman, 1951) Pm(61) would clearly fulfill the latter. In this case one might add a small known percentage of the element in question (or one of its neighbors in the periodic table) to the finely powdered sample and observe the change of intensity of some convenient line. However, Coster and Nishina (1925) found this not always to be a reliable procedure.

To circumvent these difficulties Glocker and Frohnmayer (1925) proposed measuring the absorption edge discontinuities in the sample material and interpreting the result in terms of the percentage constitution.

Exploration of this method has resulted in some interesting applications. Indeed, the method has become so useful and successful that it has been given the name of *x-ray absorptiometry* (Liebhafsky, 1949, 1951). By this means Engstrom (1946) measured with an accuracy of 10 percent the calcium and phosphorus content of biological materials present in amounts between 10^{-10} and 10^{-11} gms. An automatic spectrometer for absorption spectrometric work has been constructed at the Dow Chemical Company (Frevel, 1948).

It is by no means necessary to use monochromatic x-rays in absorptiometry. Perhaps the greater number of applications of absorption in industry use the polychromatic radiation directly from the x-ray tube, or in some cases from the sample as secondary radiator. Industrial control processes have been in use for some time on the thickness of steel sheet in the rolling mill (Clapp and Pohl, 1948), the concentration of tetraethyl lead in gasoline (Michel and Rich, 1947; Sullivan

and Friedman, 1946), the thickness of tin plate on sheet steel (Beeghly, 1950), and many other applications which the reader will find upon examining the literature.

COMPOUND ANALYSIS

As indicated earlier in this report a crystalline substance is made up of a regular array of atoms or molecules. The number of possible fundamental arrangements is fairly small and has been the subject of extensive study by crystallographers

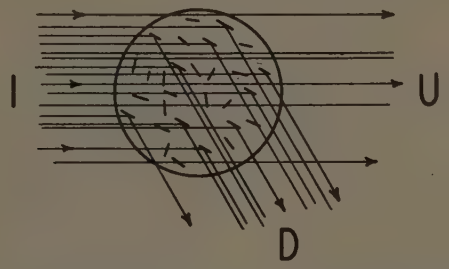

FIGURE 7. Diffraction of a monochromatic x-ray beam by randomly oriented microcrystals of a finely powdered sample. One of the possible diffraction directions is shown. Notice that all effective crystal planes are parallel. I, incident parallel beam; U, undiffracted beam; D, diffracted beam.

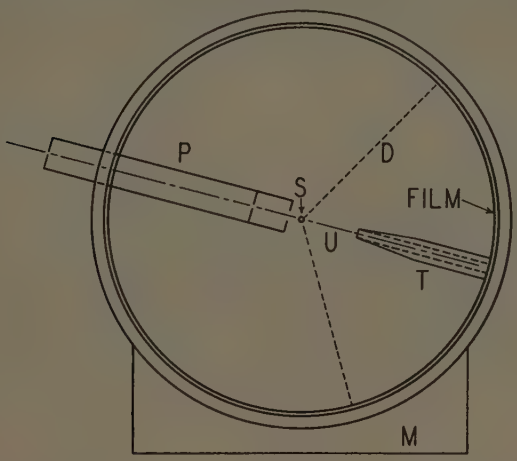

FIGURE 8. Schematic diagram of powder diffraction camera. P, pinhole system; S, powdered sample; U, undiffracted beam; D, diffracted beam; T, trap for the strong undiffracted beam; M, mounting pad. The photographic film is placed around the inside of the camera.

(Clark, 1940). In any one of the fundamental arrangements layers of atoms or molecules can be discerned in various directions in the crystal, the distance between layers depending on the particular direction chosen. An instructive comparison is furnished by the standard orchard arrangement (or corn, for the middle West reader!). If one stands in the middle of the orchard he can see rows of trees in selected directions, the distance between rows depending on the particular direction chosen.

It is a remarkable fact that a particular chemical compound, upon crystallizing, characteristically does so in one or sometimes two of the possible arrangements; and even though two different compounds may choose the same arrangement they always have different values of the atomic layer separations. Hence an identification in an unknown crystalline sample of the type of arrangement and a measurement of the so-called lattice parameters would provide complete identification of the chemical compound. However, as we shall see, it is not necessary to gather even this much information to identify the compound uniquely.

If, now, a monochromatic beam of x-rays is incident upon the crystal, reflection of the beam will occur whenever the angle of incidence on one of these atomic planes satisfies Eqn. 1. To get all the various possible reflections the crystal would have to be oriented in a number of different directions. The Debye-Scherrer method of x-ray crystal analysis uses the sample in finely powdered form, and for this reason is also known as the powder diffraction method (Clark, 1940). In any position of the crystalline mass there will always be some of the randomly oriented minute crystals in just the right position to reflect the incident monochromatic beam of x-rays from any given set of parallel atomic planes. Figure 7 shows a sample diffraction on a magnified scale and figure 8 gives the essential arrangement of a powder camera with photographic registration. The powder diffraction pattern of molybdenum is shown in figure 9.

FIGURE 9. Powder diffraction pattern of molybdenum metal taken with Fe Kα radiation. The Kβ has been eliminated by suitable filtering of the x-ray beam. (Courtesy of Battelle Memorial Institute, Columbus, Ohio.)

The question may occur to the reader as to just why the powder method is adopted for most analytical work. The reasons are several. The powder method is a simple experimental technique with uniquely interpretable results. In perhaps the majority of cases the sample is presented to the analyst in powdered form. If not, the sample can usually be powdered by grinding or filing. However, in those cases where the sample must not be disturbed single crystal techniques may be used.

Prior to 1938 if one wished to use powder diffraction patterns for chemical analysis he had to identify the crystal type from the pattern, work out the lattice parameters and then chemically identify the crystal from tables of lattice constants worked out by crystal analysts. In that year, Hanawalt, Rinn and Frevel (1938, 1944) published the first table listing not only the grating spaces corresponding to the several lines in the pattern but also their relative intensities (Sproull, 1946). These authors showed that only a moderately accurate measurement of the d values of the three most intense lines in a given pattern is enough to allow unique chemical identification from their tables. Since that time the number of compounds listed by American Society for Testing Materials has grown to approximately 5000.

In the early 1940's Dr. Herbert Friedman of the Naval Research Laboratory investigated the application of the Geiger Counter to the direct measurement of powder diffraction patterns with excellent results. This work subsequently led to the development of the North American Philips Geiger Counter Spectrometer through the cooperative efforts of Dr. Friedman and the Company's laboratory. A picture of this unit is shown in figure 10. It uses the Bragg spectrometer principle with a powdered crystalline reflector, which, of course, is the sample under investi-

gation. We are prepared now to consider this case more fully. In figure 11, FS is the extended focal spot of the x-ray tube giving monochromatic radiation. Two defining rays are shown of the radiation from FS which gets through the fairly fine slit. Notice that all the radiation from the target which gets through the slit is confined within the angle α. Beyond the slit the rays spread out and bathe the powdered sample plate PS. Those rays which arrive near A are sure to find small crystals oriented at the Bragg angle and will be reflected. The same is true at B and indeed in every region of PS receiving radiation in the angle α. The

FIGURE 10. North American Philips Geiger Counter X-Ray Spectrometer. The vertical scanning Geiger counter is seen at the right. Mounted for simultaneous use are the Weissenberg Goniometer and two powder diffraction cameras.

reflected radiation from all parts of the plate proceeds to a "focus" at F symmetrical with the slit S. We place a slit at F and allow all x-rays passing through to enter the ionization chamber. In this case, then, the position of the powdered crystal plate is of only secondary importance and we measure carefully the angular position of the ionization chamber slit. This gives us 2Θ, or twice the Bragg angle.

The Philips instrument has the advantages of excellent sensitivity, a permanent record traced out on an automatic chart recorder, very accurate intensity measurements, flexibility and speed.

Here, however, we must put in a word of caution. So long as we are interested strictly in chemical qualitative and quantitative analysis in the ordinary sense the

Geiger Counter Spectrometer is doubtless unsurpassed. However, the broader aspects of chemical analysis, in which we may be interested in problems such as particle size (especially if it is large), orientation, work hardening of metals, and so on, have special requirements which are oftentimes answered far better by the photographic film (Clark, 1940). We see that the Geiger Counter is a special tool which answers some needs admirably but offers no panacea.

There is under construction at the present time in the author's laboratory an x-ray spectrometer of modified design, which may have some application in x-ray compound chemical analysis (Broussard, 1950). The arrangement is shown schematically in figure 12. It is seen to be essentially a "double crystal" spectrometer. The characteristic x-rays from the copper target are reflected by the

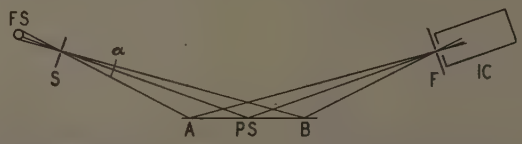

FIGURE 11. Schematic diagram of Bragg powdered crystal spectrometer. FS, focal spot of the x-ray tube; S, source slit; PS, powdered sample spread out on glass plate; F, focal line slit; IC, ionization chamber or Geiger Counter.

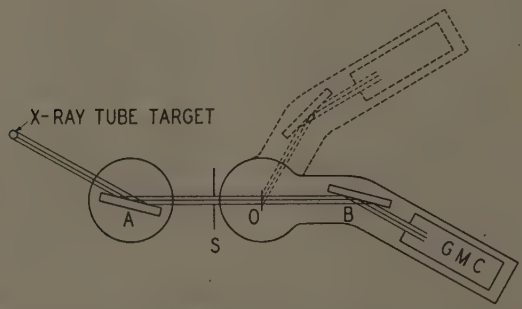

FIGURE 12. Double crystal powder diffraction spectrometer. X-ray tube target to give strong line radiation (e.g., copper); A, B, large single crystals (e.g., calcite or rock salt); S, defining slit to eliminate Kβ; O, powdered sample; GMC, Geiger Counter.

first crystal A to the sample under investigation situated on the axis O. Actually, because of the difference in Bragg angle only the K$\alpha_{1,\,2}$ radiation will reach the sample, the Kβ radiation being eliminated by the shield S. The second crystal B and the Geiger counter are mounted to move together about the axis O and to be so related that any Cu Kα radiation reflected by B will enter the Geiger Counter. The spectrometer is designed to function in the so-called "parallel" position (Compton and Allison, 1934). As the arm is made to travel around, then, by means of a synchronous motor drive, the detector will record in succession the diffracted beams, such as the dotted one in figure 12. The resolving power of this spectrometer is extraordinarily high—of the order of 10,000 as a maximum—and the line widths are correspondingly small. Any abnormally large line widths will indicate a small average crystalline size for the sample. Indeed, a line width measurement can be interpreted in terms of average particle size.

Because of the extremely narrow lines their position may be determined with great accuracy. This will allow studies on the minute changes produced in the grating spaced under certain conditions, such as the alloying of metals, intra-

granular elastic distortion produced by external stresses, and the like. This equipment should also be applicable to the study of liquid sample diffraction, and diffraction by organic substances. The particular geometry used will all but eliminate difficulties due to fluorescence of the sample.

In powder diffraction work one is always limited by the available intensity of x-rays from the source. Recently, a very ingenious cathode designed by Heil and Ebers (1950) has been adapted to the production of x-rays (Shaw and Soules, 1951). The advantage of this cathode is that it gives extraordinarily intense electron emission in a parallel beam of small cross-section. The beam is approximately 2 mm. in diameter and up to 500 ma. Work is being started to use the cathode in a rotating anode, water cooled x-ray tube designed to give a maximum of 500 ma into the focal spot (7 mm x 10 mm) at 30 KV target potential. This makes a continuously operating x-ray tube of approximately 15 KW target dissipation. The x-ray intensity increase over standard diffraction tubes in use now will be a factor between 10 and 20. The tube, of course, will be continuously pumped and many technical difficulties must be solved before it can function.

X-ray analysis has become a standard technique to be considered on a level with other analytical techniques. Its range of application is still limited, partly because of the fact that time is required to explore the possibilities and partly by instrumental limitations. Problems of the latter type are being attacked energetically by many researchers. The needs are quite clear and one may expect that instrumentation will continue to improve.

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ANALYTICAL APPLICATIONS OF MASS SPECTROMETRY

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In the early 1800's, William Prout, an English physician interested in chemical research observed several whole number atomic weights. He hypothesized a whole number of atomic weight system with all atoms being aggregates of hydrogen atoms. Prout's theory was refuted by the failure to obtain weight rectification of any of the elements having chemically determined consistent fractional atomic weights. The concept of mixed atomic masses in an element was considered ridiculous, yet it was not until the positive ray parabola research by J. J. Thompson in 1907 that experimental proof established all atoms of an element at approximately equal mass. By 1910, study of radioactivity and positive ray parabolas indicated that particles of nearly identical chemical activity could have definite mass differences. F. W. Aston is credited with proving the existence of stable isotopes in 1919 with his first mass spectrograph.

During the 1920's, mass spectrographs became popular university research tools. By the 1930's, introductory college physics texts included the simple theory of the mass spectrograph. The short twenty-one years between Aston's invention and the industrial applications of mass spectrometers indicates the value of this tool and illustrates the progressive technical leadership in industry.

The single focusing mass spectrometer is most common in analytical usage. The two principle models commercially available are the 180° magnetic deflection instrument built by the Consolidated Engineering Corporation and the 60° magnetic deflection instrument built by the General Electric Company. In both instruments, the gas or vapor sample is ionized by an electron beam and the positive ions are accelerated by an electric potential, deflected by the magnet and collected one mass at a time. The charge carried by the ions to the collector plate establishes a current in a high resistance between the collector and ground. This signal is amplified and recorded as a measure of the intensity of the ion beam for each mass scanned. The mathematical expression for mass sorting is derived from the two equations:

$$(1) \qquad E\,e = \tfrac{1}{2}\,mv^2$$

$$(2) \qquad Hev = \frac{mv^2}{R}$$

where e = charge on the ion, m = mass of the ion, v = velocity of the ion, R = radius of ion path in the magnetic field, E = accelerating potential, and H = magnetic field strength.

Equations (1) and (2) are combined to eliminate the velocity term and result in the expression

$$(3) \qquad m/e = H^2\,R^2/2E$$

In the mass spectrometer, the radius of the ion beam path is fixed and the mass range is scanned by varying either H or E. Greater resolution for extremely accurate mass determination is obtained by double focusing instruments that combine electrostatic deflection with magnetic deflection to give optimum focusing of the ion beam. Mass spectrometers are operated at pressures of 10^{-6} mm Hg or less to assure the predominance of primary processes in ionization and to make the mean free path of the ions greater than the path length in the instrument.

In analytical practice, the gas or vapor sample is placed in a large expansion volume at a known pressure and passes into the ionization chamber through a small leak.

Early analytical mass spectrometry was concerned with the identification and abundance of isotopes. A few years later, investigators used mass spectrometry to study molecular dissociation and ionization under electron bombardment. The wartime requirement for large quantities of aviation quality gasoline stimulated the development of complex petroleum processes and a parallel technical advance in analytical control. Thus, the commercial mass spectrometer was developed and became essential to the petroleum industry for quantitative analysis and process control (Washburn, Wiley, and Rock, 1943).

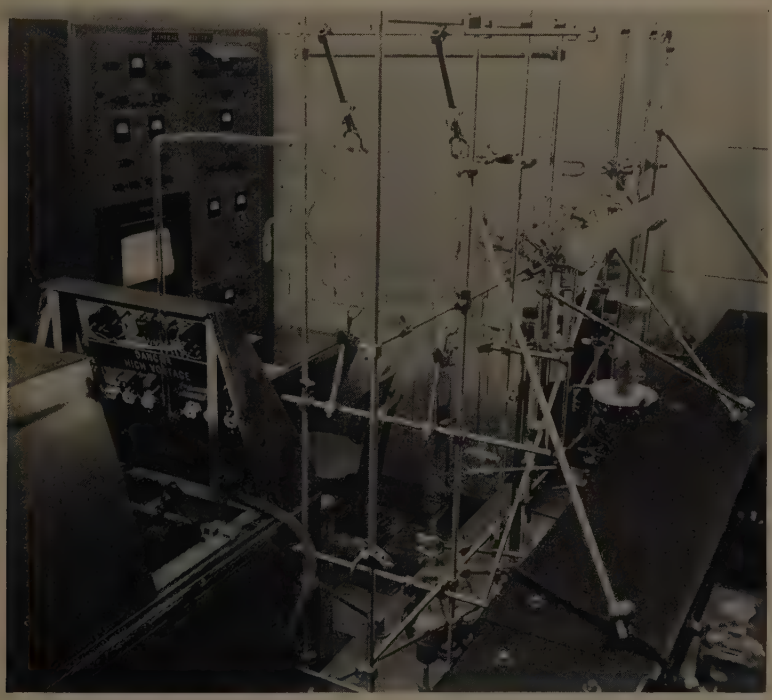

FIGURE 1. Photograph of the B. F. Goodrich Company Research Center General Electric Instrument. The spectrometer tube rack is in the lower left, the control cabinet is in the background, and the locally built sample system is shown at the right.

By using ionizing electrons with energies well above the dissociation energies for chemical bonds, the mass spectrometer records cracking patterns containing nearly all possible segments of the molecule. Nearly all molecules including isomers have cracking patterns that may be used qualitatively for identification and in most cases quantitatively for analysis in mixtures. Table 1 shows the cracking pattern of a commercial grade acetone. The mass 31 peak is rearrangement ion of CH_3O^+ form and is commonly found in oxygenated compounds. Cracking patterns differ somewhat from instrument to instrument, and even change with time for the same instrument due to uncontrolled factors. This necessitates calibration runs with pure compounds for every instrument for quantitative

analysis. For qualitative application, over 500 mass spectral data sheets have been published by the American Petroleum Institute Research Project No. 44.

The cracking pattern offers qualitative information to an experienced operator. Indications of compound purity are easily obtained by comparison of patterns. Often impurities may be recognized by the presence of peaks not consistent with the sample examined. Familiarity with masses and natural abundance of isotopes

TABLE 1

Cracking pattern of acetone at 70 volts ionization energy

m/e	Intensity	Remarks
12	0.43	C^+
13	1.14	CH^+
14	4.46	CH_2
15	25.73	CH^+_3
16	0.48	O^+ and rearrangement to CH^+_4
17	0.31	OH^+
18	1.22	H_2O^+
19	0.25	Doubly charged mass 38 ion
19.5	0.14	Doubly charged mass 39 ion
20	0.12	Doubly charged mass 40 ion
24	0.18	C^+_2
25	1.02	C_2H^+
26	4.41	$C_2H_2^+$
27	6.54	$C_2H^+_3$
28	8.99	CO^+
29	2.82	Isotope peak and rearrangement to COH^+
30	0.09	Isotope peak
31	0.47	Rearrangement to CH_3O^+
36	0.52	C_3^+
37	1.74	C_3H^+
38	2.14	$C_3H_2^+$
39	4.08	$C_3H_3^+$
40	0.82	$C_3H_4^+$ and C_2O^+
41	1.82	$C_3H_5^+$ and C_2HO^+
42	6.70	$C_3H_6^+$ and $C_2H_2O^+$
43	*100*	$C_2H_3O^+$ (Base Peak)
44	2.58	Isotope peak and rearrangement $C_2H_4O^+$
45	0.26	Isotope peak
50	0.19	Impurity
51	0.47	Impurity
52	0.27	Impurity
53	0.42	Impurity
54	0.05	$C_3H_2O^+$
55	0.29	$C_3H_3O^+$
56	0.04	$C_3H_4O^+$
57	0.79	$C_3H_5O^+$
58	26.48	$C_3H_6O^+$ (Parent peak)
59	0.90	Isotope peak
60	0.05	Isotope peak

often permits identification of certain elements in a compound and may indicate the number of such atoms present. In some cases, structural characteristics are determinable together with indications of molecule stability.

The majority of organic analyses done with mass spectrometers are quantitative. These require a knowledge of components present and a cracking pattern of each component. This is the type analysis important to the petroleum industry. Special computers have been built to permit the analysis of mass spectral data of petroleum fractions for twelve or more components in a matter of minutes. Speed

and accuracy make these analyses ideal for petroleum process control. Table 2 shows a sample cracking pattern and how it is made up of contributions to peaks from its components. Knowing the sensitivities for each component and the amount of sample introduced, the quantitative analysis of the mixture is straightforward.

During the past four years, Madorsky and coworkers (1949) at the National Bureau of Standards have reported pyrolytic techniques for the study of polymeric materials in the mass spectrometer. Their method involves the controlled thermal

TABLE 2

A sample cracking pattern showing contributions of components.

m/e	Original Sample	Propane Contribution	Original Minus Propane	Ethane Contribution	Original Minus Propane and Ethane	Methane Contribution	Remainder
12	2.15	0.28	1.87	0.34	1.53	1.51	+0.02
13	6.52	0.69	5.83	0.82	5.01	5.01	....
14	15.0	2.49	12.5	2.45	10.0	10.01	−0.01
15	66.2	8.26	57.9	3.60	54.3	54.3	
16	64.2	0.11	64.1	0.10	64.0	64.0	
26	30.4	11.5	18.9	18.9			
27	78.5	51.5	27.0	27.0			
28	164.9	83.4	81.5	81.5			
29	157.3	139.8	17.5	17.5			
30	24.4	3.07	21.3	21.3			
31	0.44	....	0.44	0.44			
38	6.54	6.54					
39	24.5	24.5					
40	3.68	3.68					
41	18.1	18.1					
42	8.22	8.22					
43	32.4	32.4					
44	41.0	40.9	0.01	....	0.01	....	0.01

degradation of polymers in vacua and the immediate immobilization of the reactive fractions by cold trapping. The five fractions collected in the process are:

1. Non-volatile residue.
2. Wax-like residue volatile at pyrolysis temperature.
3. Low volatility liquid.
4. High volatility liquid.
5. Fraction gaseous at liquid nitrogen temperature.

Most of the reported study has involved the liquid fractions. The pyrolytic technique for the study of polymers offers definite polymer identification, indicates purity, and offers promise in the investigation of correlation between structural formulae and physical and chemical properties.

Preliminary success has been reported in the study of reaction intermediates by means of a mass spectrometer (Eltonten, 1947). By coupling the instrument directly to a reaction vessel, the presence of short-lived radicals has been demonstrated. Some of the lower mass radicals have been identified and proven to be a product of the reaction. Operation of the reaction vessel over a wide temperature range has shown correlation between ion intensities and the thermal cracking of hydrocarbons. This technique is extremely sensitive—capable of detection of about five parts per million of free radical concentration. There is promise of successful investigation of high speed reactions such as flame study by probing the reaction area with a mass spectrometer.

In a discussion of recent developments in mass spectrometry at the Buffalo Instrument Conference (1950), M. G. Inghram stated that sensitivities up to 10^4 times that of ordinary wet chemical methods were possible in inorganic mass spectral analysis. Isotope dilution techniques have been established for quantitative analysis of low volatility inorganic materials. Similar methods have been used to determine reactions of impurities in slow neutron induced reactions. Daughter isotopes of different mass are added before a reaction and isotope abundance comparison with undiluted reaction material yields quantitative results (Hayden, Reynolds, and Inghram, 1949).

Either short half-lives or limited availability of radioactive isotopes of such common elements as hydrogen, nitrogen, potassium, and oxygen makes mass spectral stable isotope tracer study important to the chemist. As yet, low enrichments and limited supplies of stable isotopes have restricted mass spectral tracer studies. All isotopes of an element are not strictly identical in chemical behavior since bond strength is influenced by atomic mass. This difference is most notable in the case of hydrogen and deuterium and decreases with increasing mass. The dissociation of bonds between light isotopes are more probable than the dissociation between heavier isotopes. For example, the dissociation of the C^{12}-O^{16} bond is about 3.5% more frequent than for the C^{12}-O^{16} bond after allowance is made for the relative abundances of C^{12} and C^{13} isotopes. These variations are noted in thermal dissociation as well as in electron dissociation in the mass spectrometer.

Any routine stable isotope enriched tracer study will require compensation for isotope effects in cracking patterns. Since these effects will increase in complication with the complexity of the molecules and the number of tracer atoms, isotope exchange with less complicated compounds will be applied.

Stable isotope tracer experiments are particularly useful in biological research. Compounds labeled with N^{15} have been used to investigate incorporation of the compounds in proteins. Rates of protein synthesis in different organs are measured. The metabolism of carbohydrates has been investigated by using deuterium and C^{13} stable isotopes as well as radioactive P^{32} and C^{14}. Tracer compounds become quite diluted in most experiments. This means that success is generally limited to elements having low natural abundance of the tracer isotope. Stable isotope tracer work becomes difficult, if not impossible, in the case of elements such as iron and chlorine. Fortunately, in most of these cases there are now available radioactive isotopes of satisfactory half-life. An excellent account of the use of stable and radioactive isotopes in biological research is in *Isotopes in Biology and Medicine* by nineteen leaders in fields of physics, chemistry, physiology, and medicine; The University of Wisconsin Press (1948).

The mass spectrometer has proven itself in the petroleum industry as an essential analytical and process control instrument. Mass spectrometers are currently entering such diverse industries as chemical, rubber, glass, air products, meat packing and food processing, metal, and aircraft products. The manufacturers of mass spectrometers are conducting extensive instrument research and development programs. The Bennett type radio frequency mass sorting tube and the ion resonant tube are promising new developments applicable to the lower mass region for leak detection and process control.

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RADIOACTIVE METHODS OF ANALYSIS

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The application of radioactive materials to the study of the problems in biology, physics, chemistry, and industry is growing rapidly. This is mainly because of the utility of radioisotopes as analytical tools.

Radioactive methods of analysis are particularly useful for the detection and determination of trace quantities of materials and for the measurement of larger quantities in complicated systems or samples.

PROPERTIES OF RADIOISOTOPES

Radioisotopes are unstable atoms which decay by the emission of radiation, alpha, beta, or gamma rays.

Radioisotopes have two properties which make them important analytical tools: (1) They behave chemically the same as the isotopes of the elements, and (2) Their radiation indicates their position and quantity.

$$N = N_o e^{-\lambda t}$$

The rate of distintegration and the energy of the radiation emitted are characteristic functions of a given radioisotope. The rate of decay is exponential, where N = the number of atoms at time t, N_o = the number of atoms at time t_o, and λ = the characteristic decay constant.

The rate of distintegration is conveniently described in terms of a half-life, that is, when

$$N = \frac{N_o}{2},$$

$$t_{1/2} = \frac{0.693}{\lambda}.$$

The energy of the radiation emitted by a radioisotope may be expressed in terms of the absorption of the energy by matter. The absorption of gamma rays and, in some cases, of beta particles may be represented by the formula,

$$I = I_o e^{-\mu d},$$

where I = the intensity at thickness d, I_o = the original intensity, μ = the absorption coefficient, and d = the thickness of absorber.

When, $I = \frac{I_o}{2}$

$$d_{1/2} = \frac{0.693}{\mu}$$

The energy of the radiation may be defined in terms of the half-thickness, $d_{1/2}$, usually expressed in mg/cm^2.

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ISOTOPIC TRACING

Radioisotopes are used most frequently as isotopic tracers. A small, known quantity of a radioactive-labeled form of the compound to be traced is mixed with the experimental sample at the beginning of the work. The labeled form of the compound will behave the same as the nonactive compound during treatment of the sample. The measurement of the radiation from any fraction of the sample is proportional to the quantity of the compound present.

Examples of the conditions under which radioisotopes are useful as isotopic tracers are:

(1) The quantity of the material to be traced is very limited. For example, it is possible to detect as little as 10^{-12} grams of several of the radioisotopes. Some of the most interesting examples of ultramicrochemical work with tracers are found in the description of the isolation and identification of new elements, such as technetium, promethium, curium, and californium. None of these elements have been found in nature since they are radioactive and have half-lives which are short compared to the age of the earth.

In the production and identification of element 98, californium, Street, Thompson, and Seaborg (1950) worked with only a few thousand atoms of the new element. In addition, the investigation was complicated by the fact that californium-244 has a half-life of about 45 minutes. Prior to the experimental work, it was necessary to predict some of the nuclear and chemical properties of californium so that satisfactory chemical separation procedures and detection instruments could be used. The chemical properties were predicted by comparison of the lanthanide rare earth series of elements to the actinide series in which californium would fall. On this basis, element 98 should be an "eka-dysprosium." It should have a stable plus-three oxidation state and behave like a rare earth. Ion-exchange procedures which are effective for the separation of the lanthanide rare earths should be useful for the isolation of element 98.

It was believed that californium-244 would have a half-life of one-half to several hours and would decay by the emission of alpha particles with an energy of 7.0 to 7.3 Mev. The chemical separation procedure was designed so that a half-life as short as 20 minutes could be detected. The californium was produced by bombarding curium-242 with 35-Mev helium ions in the 60-inch cyclotron of the Crocker Radiation Laboratory. The target was dissolved in an acid and the metal ions were adsorbed on a cation-exchange resin. The separation of the cations from the resin was carried out by selective elution with a citrate solution. By comparison with the elution of the lanthanide series, for which the order of the removal from the resin is dysprosium, terbium, gadolinium, and europium, it had been predicted that californium would be eluted first and then berkelium, curium, and americium. The predictions were found to be valid. An alpha activity with an energy of 7.1 Mev and a half-life of 45 minutes was found. Although the new activity was contaminated by some curium-242, it was possible to distinguish between the two activities since the alpha particles from curium have a lower energy, about 6 Mev. Tracer experiments were conducted with the new element to study some of its chemistry. Oxidation experiments showed that californium could not be oxidized to the four or six valent states.

Radioactive microchemical methods are important in industrial research. Insecticides which have been synthesized with radioactive sulfur-35 have been used to measure the quantity of the insecticide which was taken up by insects and the plant material (Anonymous, 1950).

Another example is the determination of the separation efficiency of chemical processes. In the case in which it is required that a product of high purity be produced, radioactive forms of the impurities may be used to measure the contamination of the product.

(2) The material to be traced is associated with other materials which are difficult to separate from one another. For example, the metals hafnium and

zirconium are extremely difficult to separate. Through the use of either radioactive hafnium-181 or zirconium-95, the analysis may be made without a tedious separation.

(3) A material is to be located with reference to the position of other materials in a sample. The investigation of the segregation of trace elements in an alloy is an example of this application. Autoradiographic techniques in which the position of the radioactivity is found by the exposure of a photographic emulsion are frequently used.

(4) The sample must be analyzed nondestructively or in place. Radioactive dyes (Griffin, Goland, and Chamberlain, 1950) such as diiodofluorescein have been employed for the diagnosis of intracranial lesions. The dye is labeled with the gamma-emitting radioisotope iodine-131. Detection of the location of the dye in the diseased tissue in the brain is accomplished through the use of a directional gamma ray detector.

(5) A determination must be made rapidly before some further change occurs. Radioisotopes might be used to control a process in which several operations are being done automatically. For example, in the treatment of a fabric, traces of the soap used in the washing operation may interfere with the dyeing operation. A radioisotope-labeled soap could be used to insure that the soap had been removed prior to the dyeing of the fabric.

(6) The diffusion or reaction of a material with itself must be measured. It is possible to determine the self-diffusion coefficients of the metals. The kinetics of isotopic exchange reactions may be studied. For example, the rate of exchange between different oxidation states of the same metal may be measured.

ANALYSIS BY ISOTOPIC DILUTION

One of the most useful methods of analysis with radioactive materials is isotopic dilution. The method is based upon a comparison of the specific activity of a radioactive-labeled material before and after dilution with a nonactive form of the same material. Isotopic dilution is of particular advantage for the determination of a compound which is difficult to separate quantitatively from a sample.

The method may be illustrated by an example: (1) Assume a sample which contains an unknown A grams of X, (2) Mix B grams of X having a specific activity of S_B counts per minute per gram into the sample, (3) Separate a portion of X in pure form, and (4) Determine the specific activity of S_{AB} of this portion of X. Then the various quantities may be related through the equation,

$$BS_B = (A + B)\, S_{AB},$$

and A calculated by,

$$A = B\left(\frac{S_B}{S_{AB}} - 1\right).$$

The technique may also be used to analyze an unknown radioactive sample. In this case, the sample is diluted with a portion of a nonactive form of the compound.

The specific activity before S_A, and after, S_{AB}, dilution are measured. The equations involved are,

$$AS_A = (A + B)S_{AB},$$

$$A = B\frac{S_{AB}}{S_A - S_{AB}}.$$

RADIOMETRIC ANALYSIS

The process of analyzing a nonactive component by combination with a radioactive isotope is known as radiometric analysis

For example, radioactive silver might be used to determine a small quantity of chlorine in a sample. The radioactive silver, Ag*, would combine with the chloride ions,

$$\underset{107.9}{Ag^{*+}} + \underset{35.46}{Cl^{-}} \longrightarrow Ag^{*}Cl,$$

to form active silver chloride. The silver chloride could be separated from the solution by centrifugation or by collection on a precipitate of ferric hydroxide.

The weight of silver used could be calculated from the specific activity, S_{Ag}, of the silver added and the activity, A_{AgCl}, of the silver chloride:

$$\text{Weight of Ag} = \frac{A_{AgCl}}{S_{Ag}}.$$

The weight of the chlorine could be found from the stoichiometry of silver chloride:

$$\text{Weight of Cl} = \frac{35.46}{107.9} \times \frac{A_{AgCl}}{S_{Ag}}.$$

ANALYSIS WITH NEUTRONS

Several methods of analysis have been developed utilizing the absorption and scattering of neutrons by matter. Since neutrons are neutral particles, they can approach and enter a nucleus. High neutron energies are not required because a neutron does not have to overcome repulsive electrical forces. The cross section of a nucleus is the effective area presented to a neutron. The total cross section is made up of two components, an absorption and a scattering cross section. In the case of absorption, a nucleus takes on a neutron, and another form of radiation, such as an alpha particle or a photon of gamma rays is emitted. Scattering consists of a nucleus receiving a neutron and emitting a neutron.

Although neutrons are particles, they behave with wave-type properties. The wavelength, λ, of a moving neutron is defined as,

$$\lambda = \frac{h}{mv},$$

where h is Plank's constant and m and v are the mass and velocity of the neutron.

The best source of neutrons is a nuclear pile which produces 10^{10} to 10^{12} neutrons per square centimeter per second. A cyclotron which uses deuterons and a beryllium target may produce 10^{8} to 10^{9} n/cm^2/sec.

Portable neutron sources which consist of an alpha- or gamma-emitting radioisotope and beryllium or boron are available. The neutrons are produced by the action of the radiation on the beryllium or boron. Portable sources may produce about 10^{3} to 10^{5} n/cm^2/sec. Their use is limited because of their lower yield of neutrons.

Neutron Activation

Samples of some materials may be analyzed by neutron activation (Taylor and Havens, 1950). The sample is placed in a flux of slow neutrons. Some of

the atoms of the sample absorb neutrons and become radioactive isotopes. A determination of the half-life and energy of the radiation identifies the radioisotope present. The quantity of the original material present may be calculated.

The rate of production of the radioactive atoms is given by the difference between their rate of formation and their rate of decay:

$$\frac{dN^*}{dt} = N\sigma f + \lambda N^*, \quad (1)$$

where N^* = number of active atoms, N = number of original inactive atoms, σ = atomic activation cross section, f = flux of neutrons, λ = decay constant for N^*, and t = time.

Integration and substitution of the activity, $A = N^*\lambda$, gives

$$A = N\sigma f\,(1 - e^{-0.693t/t_{1/2}}) \quad (2)$$

where $t_{1/2}$ = half-life of N^*. When t is much greater than $t_{1/2}$, the saturation activity in the sample is approached and the formula simplifies to

$$A = N\sigma f. \quad (3)$$

If the measurement of the activity is made at a time t_1 after neutron irradiation is ended, the activity is related to the original activity in the following manner:

$$A_{t_1} = Ae^{-\lambda t_1}, \quad (4)$$

where A_{t_1} = the activity at time t_1, and A = the activity at the end of neutron irradiation.

By substituting the expression for A in equation (3) into equation (4), we obtain

$$A_{t_1} = f\sigma Ne^{-0.693t_1/t_{1/2}}. \quad (5)$$

Since $N = 6.02 \times 10^{23} \times \frac{g}{M}$, the following formula for the weight, g, of the material in the sample is obtained:

$$g = \frac{A_{t_1}M}{6.02 \times 10^{23} f\sigma e^{-0.693t_1/t_{1/2}}}. \quad (6)$$

Now, if t is not much greater than $t_{1/2}$, the formula needs to be corrected, since the activity of the sample was not at saturation:

$$g = \frac{A_{t_1}M}{6.02 \times 10^{23} f\sigma\,(1 - e^{-0.693t_1/t_{1/2}})\,e^{-0.693t_1/t_{1/2}}}. \quad (7)$$

For accurate work, the absolute activity of the sample must be determined by comparison with a standard. The counting efficiency of most nuclear detectors is only about 5 to 35 percent. It is very difficult to determine absolute disintegration rates. Examination of formula (7) shows that those elements with large cross sections and short half-lives are the easiest to detect. Table 1 gives the atomic activation cross section, the radioisotope produced, and the half-life of the radioisotope for several elements. The minimum detectable quantity of the

TABLE 1

List of Cross Sections (Taylor and Havens, 1950)

Element	Cross Section, Barns	Radioisotopes	Half-Life*
Ir	285	Ir^{192}	70 d
Ag	48	Ag^{110}	24 s
Cl	40	Cl^{36}	2×10^6 y
Co	22	Co^{60}	5.3 y
Mn	11	Mn^{56}	2.6 h
I	6.25	I^{128}	25 m
Ni	2.9	Ni^{59}	16 y
Cu	1.99	Cu^{64}	12.8 h
Cr	0.49	Cr^{51}	26.5 d
Fe	0.15	Fe^{55}	4 y
Mg	0.0054	Mg^{27}	10.2 m

* s = seconds, m = minutes, h = hours, d = days, y = years.

elements may be calculated with the equation developed above. The formula is simplified if it is assumed (1) That the time of irradiation is long compared to the half-life of the radioisotope, and (2) That the activity measurement is made immediately after irradiation.

$$g \times 10^{-12} = \mu\mu g = \frac{kA_{t_1}10^{12}}{6.02 \times 10^{23}\, f\sigma \times 10^{-24}},$$

where k = counting efficiency = 25 percent, A_{t_1} = 100 counts per second, f = 10^{12} u/cm²/sec., and σ = cross section in barns.

The equation reduces to

$$\mu\mu g = \frac{41.5 \times M}{\sigma}.$$

The calculation of the minimum detectable quantities of a few elements according to the previous assumptions is shown in table 2.

TABLE 2

Element	$\mu\mu g$	σ	M
Ir	1.5	285	193.1
Mn	208	11	54.93
Cu	1,320	1.99	63.57
Mg	187,000	0.0054	24.32

Samples in which two components are activated by neutron irradiation may be analyzed. The analysis becomes quite difficult if more than two active components are present. In a two-component system, the elements present may be detected

by the characteristic half-life and energy of the radiation of the radioisotopes formed. In order to distinguish between the two elements, it is necessary that the half-lives and energies of the radioisotopes be quite different. The half-lives of the two radioisotopes may be determined by plotting the time versus the log of the activity of the sample, Figure 1. The shorter half-life, a, is found by drawing a tangent to the curve at the time, t_o. The longer half-life, b, is measured from the tail of the curve after all of the isotope, a, has essentially decayed. In this case, the half-lives of a and b were found to be 0.55 and 5.0 days, respectively.

The activity of the two components at t_o may be found. In the case of b, this may be found by extrapolation of the tail back to t_o. The activity of a is then equal to the difference between the total activity and that of b at t_o. These activities may be used to calculate the quantity of a and b in the sample. The energy of the radiation from the two radioisotopes may be measured in a manner

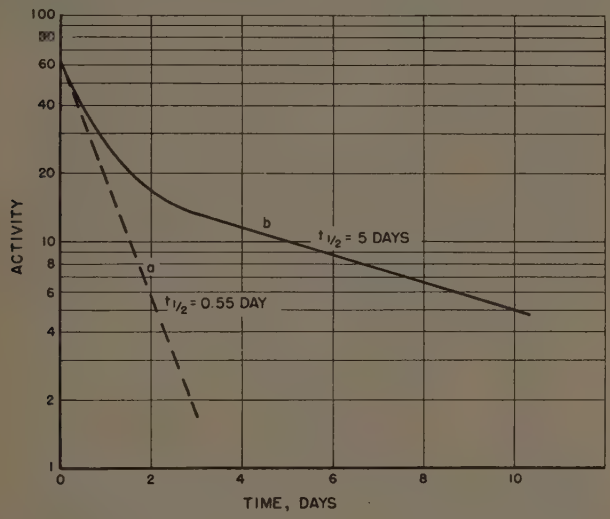

FIGURE 1. Determination of half-lives in a two-component system.

similar to that of the half-lives. An absorption curve is plotted by measuring the activity of the sample through different thicknesses of an absorber such as aluminum or lead (fig. 2). The half-thicknesses are determined in the same manner as the half-lives. The energies are found from curves or data on the half-thickness of various energies of the beta or gamma radiation being measured.

Neutron Absorption-Heterogeneous Beam

It is possible to determine small quantities of some elements which have large neutron absorption cross sections by the measurement of the transmission (Muelhause and Thomas, 1950) of a beam of neutrons, having energies up to 50,000 electron volts. The intensity of the beam is measured with and without the sample interposed.

The transmission data are related to the cross sections of the two components as shown in equation (1):

$$n_1 \sigma_1 + n_2 \sigma_2 = \ln \frac{I_n}{I}, \quad (1)$$

where n_1 and n_2 = the numbers of atoms of elements 1 and 2, respectively, σ_1 and σ_2 = the cross sections of elements 1 and 2, respectively, I_o = the intensity of the neutron beam without the sample, and I = the intensity of the neutron beam with the sample.

The mass of the sample may be expressed by:

$$m = \frac{(n_1A_1 + n_2A_2)}{N},$$

where m = the mass of the sample per cm², A_1 and A_2 = the atomic weights, and N = Avogadro's number.

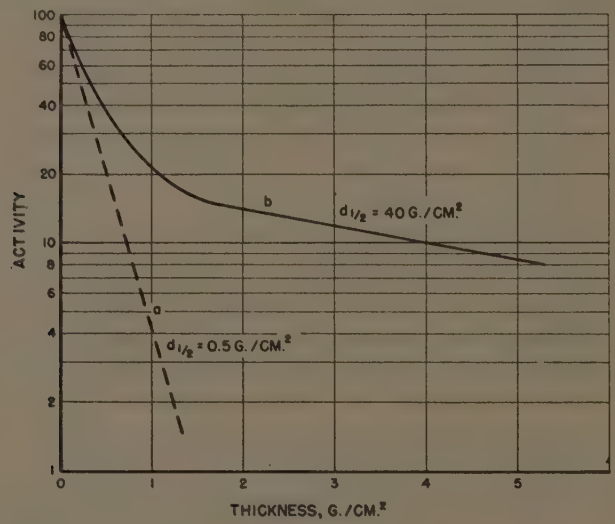

FIGURE 2. Determination of energy in a two-component system.

Combining equations (1) and (2),

$$n_1 = \frac{\ln \frac{I_o}{I} - \frac{mN\sigma_2}{A_2}}{\sigma_1 - \frac{A_1\sigma_2}{A_2}}. \quad (3)$$

An example of the use of the method is the determination of lithium in magnesium. A measurement of 5 percent lithium could be made to ±0.2 percent.

Neutron Absorption—Two Neutron Energies

In the neutron energy range of about 0.1 to 1 electron volt, cross sections vary inversely with the square root of the energy (Taylor and Havens, 1950). The composition of a two-component system may be estimated if the two elements have different cross sections at two energies.

Through the use of a neutron spectrometer, the transmission of two neutron beams of different energies through the sample may be determined. Two equa-

tions of the type indicated for the absorption of a heterogeneous neutron beam are obtained:

$$n_1 \sigma_1^1 + n_2 \sigma_2^1 = \ln \frac{I_o}{I}$$

$$n_1 \sigma_1^2 + n_2 \sigma_2^2 = \ln \frac{I_o}{I}$$

where σ^1 and σ^2 = the cross sections at the two neutron energies. These two equations may be solved simultaneously to find n_1 and n_2, the number of atoms of elements 1 and 2, respectively.

In order to make an analysis of this type, the cross sections of the two elements at the two energies must be known. Examples of application of the method are the estimation of manganese in iron and aluminum and tantalum in columbium.

Neutron Resonance Absorption and Scattering

Many of the elements exhibit a marked increase in their cross sections at definite neutron energies in the range of 1 to 5000 electron volts. This is known as neutron resonance absorption and scattering (Taylor and Havens, 1949). Resonance is usually associated with those elements with atomic masses over 100. Using a neutron spectrometer, it is possible to plot the transmission of a sample at various neutron energies. The observation of a resonance absorption may be used for the identification and determination of the quantity of a particular nuclear species in a sample. A few examples of analyses which may be made by resonance absorption are tantalum in columbium, hafnium in zirconium, and cobalt in steel.

Neutron Diffraction

Diffraction effects may be observed with thermal neutrons. These effects take place in accordance with the Bragg relation,

$$n\lambda = 2d \sin \theta.$$

Laue patterns may be obtained by passing a beam of thermal neutrons through a crystal. The neutrons may be used in a manner similar to X-rays. The major advantage of neutrons is that they may be used to locate the position in a crystal of the light elements, such as hydrogen, which have very low scattering power for X-rays. The diffraction pattern of neutrons must be detected by an indirect method since neutrons do not strongly affect a photographic emulsion. A thin sheet of a material having a high cross section, such as indium, is placed in front of the photographic emulsion. The radiation produced in the indium causes exposure of the film.

LIMITATIONS OF RADIOACTIVE METHODS

Radioactive methods of analysis are subject to limitations which require consideration. Foremost of these is the effect of radiation on the tissues of the human body. Specialized equipment and techniques are usually required to work safely with radiochemicals. The methods of detection of radioactivity present another limitation to quantitative measurements. Most of the common nuclear detectors do not have an efficiency greater than 35 percent. In addition, absorption of radiation may take place within the sample being measured, or secondary radiation may be produced. Care must always be exercised to correct a series of determinations on different samples which are to be compared to some standard set of conditions. Another complication of the use of radioisotopes is

that it may be difficult to introduce the radioactive material in the proper form. Although it is possible to purchase some radioisotope-labeled compounds, it is frequently necessary to synthesize the compound from some other form. In some cases, the synthesis may be difficult or impossible. In some cases, it is desirable to measure the quantity of more than one component of a system. The determination of even two radioisotopes in the same experiment becomes quite complicated.

While it is usually assumed that isotopes of the same element behave chemically the same, those of the lighter elements do not. For example, one may find a discrepancy of nearly 15 percent in the use of tritium as a tracer for hydrogen. In extremely dilute solutions, a material may have different properties than in more concentrated solutions. For example, the radioactive ions in a very dilute solution may be absorbed on dust particles and behave as colloidal particles.

In spite of these limitations, radioisotopes are powerful tools for the analyst. Radioactive methods should be considered along with other available methods of solving a particular problem. The procedure which appears to have the greatest advantage should be selected.

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Practice of Wildlife Conservation. *L. W. Wing.* John Wiley and Sons, Inc., New York. First edition, 1951. viii+412 pp. $5.50.

This clear, concise text book follows a well-organized topical outline with the result that it touches, at least briefly, on most of the varied aspects of conservation and wildlife management. Furthermore, it contains the results of a large selection of important studies on wildlife species, including those on the introduction of several exotic species into new areas.

The intimate relationship between plants and animals is well treated, and the importance of ecological studies emphasized. The writer has departed somewhat from the traditional procedure of implying basic biological principles to one of making clear statements of many concepts based upon sound biological principles.

A broad coverage of wildlife species, without neglecting or over-emphasizing any single region, makes this book of general interest to wildlife management personnel. Emphasis is such that it constitutes an excellent text for the training of wildlife technicians, and as a result fills a much needed place in the field of wildlife management not presently occupied by any other available text.

FRED H. GLENNY

General Chemistry. *John A. Timm.* McGraw-Hill Book Co., New York. Second edition, 1950. 764 pp. $4.50.

This edition represents a careful revision of the first edition (1944). The organization of topics is good: the elements are discussed in their natural families; three chapters are included on organic chemistry; and the atomic concept is introduced early. In general the book maintains the same high quality of the early edition. Several features, however, could have been improved, for example: there are only two illustrations in the first twenty pages; the first paragraph of chapter 1 refers to *the war, the Japanese* and *our bombers*; figures 4, 5, 7, 8, 9 show the atoms *separated* from each other in the molecules of hydrogen, oxygen, water and carbon dioxide; figures 134 and 135 are located in such proximity that figure 135 gives the impression of being an explanation of figure 134. The author is to be commended for including several new concepts such as electronegatinity, resonance, and the modern concept of acids and bases. All in all it is a good book.

A. B. GARRETT.

CHEMICAL EXAMINATION OF AN ANCIENT SHEET OF METAL OF UNIQUE COMPOSITION FOUND IN GREECE[1]

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Several years ago a native found in the bed of the Alpheus River in Greece a roughly rectangular sheet of metal which was evidently the flattened side of a cylindrical vessel, possibly a bucket or similar container. A repouseé design in Greek style of considerable artistic merit had been formed in the metal. This sheet of metal was brought to the attention of an archaeologist who purchased it because of its artistic value. Since the object was uniformly covered with a green patina, it was first believed to be composed of bronze or copper, but, in the course of cleaning it for the purposes of study and exhibition, the surface of the bare metal was seen to have the appearance of silver, which was puzzling in view of the color of the patina. Because of this discrepancy the writer was asked to make a chemical examination.

Visual inspection of cleaned places on the edge of the sheet showed that it was composite in structure. The sheet consisted of a layer of a reddish metal sandwiched between two layers of a white metal, and the three were firmly bonded

TABLE 1

Quantitative analysis and probable original composition of the white alloy

Elements Present	Analytical Figures, %	Probable Original Composition, %
Copper	52.0	52.8
Tin	32.5	33.0
Lead	13.6	13.8
Iron	0.4	0.4
Oxygen and other non-metallic impurities	1.5	none
	100.0	100.0

together. The reddish metal was ductile, but the white metal was slightly brittle and had a noticeably crystalline structure. In addition to the thin layer of patina on its outer surface, a slight amount of intercrystalline corrosion was evident in the white metal. Qualitative tests on minute samples of the two metals showed that the reddish metal was essentially pure copper and that the white metal was an alloy of copper, tin, and lead. For a quantitative analysis of the white metal a total sample of 100 milligrams of filings was collected from various parts of the edge of the sheet. The results of this analysis are shown in table 1 along with the corresponding figures for the probable composition of the unoxidized alloy. These data are given only through the first decimal place because of the small size of the sample and the uncertainty inherent in the method of sampling. The oxygen and other nonmetallic impurities were estimated by difference. It seems reasonable to conclude that the intended composition of the alloy was about one-half copper, one-third tin, and one-sixth lead, and that the iron is an accidental impurity.

[1]A paper presented at the Sixtieth Annual Meeting of the Ohio Academy of Science, Oxford, Ohio, April 6, 1951.

The composition of this white alloy is of considerable interest, as there appears to be no experimental evidence that any ancient or modern alloy of exactly this composition has ever been used in the fabrication of any other object. Of the ancient objects that have been analyzed only one other approaches this one in form and composition. This was a fragment of sheet metal, reported as apparently being tin plated on one side, which was found in the Crimea under circumstances that indicated an ancient Greek origin. The results of the analysis[2] are shown in table 2.

From these analytical figures and the description it is somewhat probable that this fragment of sheet metal may have been very similar in structure and composition to the object found in Greece. Possibly the sample that was taken for analysis included material from an underlying or intermediate layer of copper. This would account for the higher percentage of copper and the lower percentages of tin and lead as compared with those in the white alloy of the sheet of metal found in Greece. What was reported to be a coating of tin on the fragment of sheet metal from the Crimea may in fact have been an alloy very similar in composition to this white alloy. However, a close similarity of the two objects in structure and composition is by no means certain.

TABLE 2

Analysis of a fragment of sheet metal found in the Crimea

Elements Present	Analytical figures, %
Copper	66.80
Tin	21.70
Lead	11.36
Iron	0.09
Cobalt	0.05

The excellent state of preservation of the thin sheet of metal found in the river bed in Greece is explained by its chemical composition. Though pure copper itself is resistant to natural agents of corrosion, copper alloys containing a high proportion of tin, such as the white alloy of this object, are much more resistant. This is known from a detailed study made by Fink and Polushkin (1936) of the mechanism of corrosion in ancient bronze. They found that copper-tin alloys containing upwards of 25 percent of tin, or the components of an ordinary bronze that contain high percentages of tin, are much more resistant to corrosion than copper-tin alloys that contain lower percentages. They found that an alloy containing 25 to 26 percent of tin, the so-called eutectoid, survived in an isolated form in the mass of corrosion products resulting from the complete mineralization of the remainder of an ordinary bronze. The same high resistance to corrosion holds for copper-tin alloys that contain still higher proportions of tin; in these the alloy is composed of eutectoid and delta bronze. This high resistance to corrosion of copper-tin alloys containing high proportions of tin is also shown by the usual excellent state of preservation of Greek, Roman, or Chinese mirrors, many of which have been found with much of their original lustrous surface still intact.

The thin greenish layer of corrosion products on the surface of the object was probably formed in large part from the corrosion of the white alloy, as the copper content of this alloy is high enough to yield a sufficient proportion of copper compounds to mask the white compounds formed from the corrosion of the tin and lead

[2]Analyzed by Fellenberg and cited by Bibra (1869).

in the alloy. However, numerous isolated specks of the corrosion products of tin and lead were plainly visible in various areas on the surface. Some of the greenish patina may have been a result of the corrosion of the underlying layer of copper, for it seems entirely possible that corrosion products of copper could have diffused up between the crystals of the white alloy.

Both the composite structure of this sheet of metal and the physical properties of the component metals indicate that only one method could have been used in its fabrication. Casting seems clearly impossible, either for the thin central layer of copper or for the object as a whole. The formation of the sheet by hammering out metal of this composite structure also seems impossible because the white alloy is so brittle that such treatment would have cracked it. There are at least two indications that the central layer of pure copper was formed from a plain sheet of copper by hammering, probably by hammering it against a form bearing the design in relief. The variable thickness of the layer of copper indicates this technique. The use of pure copper rather than the more usual bronze is also an indication, since pure copper is much more suitable for such repouseé work than bronze because of its greater softness and malleability. After the design had been formed in the copper sheet by this method, the sheet was probably then coated with the white alloy by dipping the cleaned and cold copper into the molten alloy and withdrawing it. Since the melting point of this white alloy is about 300° C below that of pure copper no distortion of the copper from softening or fusion could have occurred. The white alloy probably solidified rapidly on removal from the fused alloy, and the result was a thick, uniform coating that adhered firmly to the copper. The intimate bond observed between the layer of copper and the layers of white metal indicates that this alloy was applied in a molten condition. It seems rather likely that the side and bottom were coated separately and then united in making a vessel of such composite metal, but it is by no means improbable that the copper parts were first united before coating. Regardless of the exact procedure, the coated surfaces were probably finished by tooling and polishing. In its completed state such a vessel must have closely resembled one of polished silver.

This sheet of metal is of considerable historical interest as an example of a large plated ancient object. Many plated ancient coins have been found, especially coins with a silver plate on a copper core (Campbell, 1933), but very few large plated objects have been found, or at least very few have been identified as such. That the art of plating large objects was known and practiced in Roman times, at least, is evident from some remarks of Pliny. In one place[3] he states, "A method has been devised in Gaul for plating copper objects with tin, so skillfully that they can scarcely be distinguished from silver; these are called *incoctilia*." In another place[4] he states, "When copper vessels are plated with *stagnum* they have a less disagreeable taste, and the onset of verdigris is checked." From his remarks in a preceding passage it would appear that *stagnum* was ordinarily an alloy of silver and lead. He then goes on to say, "A counterfeit *stagnum* is now made from pale bronze and tin in the proportion of two to one, and another, now called *argentarium*, from tin and lead in equal amounts." Since Roman bronze was an alloy of copper, tin, and lead, usually with a considerably higher proportion of lead than tin, the first kind of counterfeit *stagnum* was evidently an alloy containing a little more than one-third tin, about one-half copper, and the remainder lead, or in other words an alloy very similar in composition to the white alloy of this sheet of metal found in Greece. As a matter of fact, this object found in Greece may very well have been made in Roman times, for the conditions under which it was found gave no clue as to its date of manufacture or date of deposition. At any rate, the rather

[3] *Natural History*, Book XXXIV, par. 162.

[4] Book XXXIV, par. 160.

close correlation between the composition of the one kind of alloy mentioned by Pliny as being used to plate copper objects and the actual composition of the plating on this object is both interesting and significant.

It seems quite possible that various large Greek and Roman metal objects, especially vessels of sheet metal, now on exhibition in our museums, and which are now regarded as being composed of bronze or copper, may in fact be plated objects of various sorts. At least this possibility seems worthy of some investigation.

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A Source Book in Animal Biology. *Thomas S. Hall.* McGraw-Hill Book Company, Inc., New York. First edition, 1951. xv+716 pp. $10.00.

In this source book editorial comment is kept to a minimum, serving to acquaint the reader with biographical data concerning the investigator and his position in time. The eight divisions of the book, with excerpts of actual writings quoted in some detail, are arranged in chronological order as nearly as possible. The omission of many investigators, of whom one invariably thinks when considering historical backgrounds of animal biology, results from the fact that the Source Book Series, to which this book belongs, is arbitrarily limited to scientific discoveries from around the fifteenth to the nineteenth centuries inclusive.

The first division concerns the structure of animals and various attempts at showing, by systems of classification, the relationship of man not only to the lower animals, but also to other members of his own species. Developments in anatomy, gross and histological, are traced, likewise those in embryological studies.

In the second and third divisions man and the functioning of the various portions of his body and mind are lifted from the realm of the supernatural to conformity with physical and chemical laws and principles.

From spontaneous generation, through epigenesis to organ-forming germ-areas, the development of embryological knowledge is treated in the fourth division. Cellular and tissue differentiation receive consideration, pointing up evidence for evolution from a comparative study of embryos.

The fifth division deals with cytology. Microscopy furnishes the device whereby investigation reveals cellular structure leading to concepts of protoplasm, its constituent parts and the role of highly specialized cells in reproduction and sex-determination.

Division six, dealing with pathology is treated in an unusual manner in that the contributions include widely different aspects of bodily disorder—microorganisms, viruses, animal parasites, vectors, dietary insufficiencies and immunology.

Evolution and paleontology are fittingly presented together in division seven. Discussion of mechanisms and theories of heredity is related to evolutionary development.

Zoogeography constitutes the last division, with a brief discussion of earth's areas and regions and their typical faunas.

This excellent reference book should prove useful to advanced students of zoology. It is valuable to teachers of many branches of zoology, who wish to broaden their students' appreciation of the science of the present day in relationship to the past. Adversely, the inclusion of Willoughby's dissertations on the training of hunting dogs, methods of catching or shooting birds and hunting and game laws appears to have dubious value in this well-planned and well-arranged source book.

E. Eloise Whitwer.

STUDIES OF THE GENUS EMPOASCA

(HOMOPTERA, CICADELLIDAE).

XII. New Species of Mexican Empoasca

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In the preceding papers of this series upon *Empoasca*, many species have been described from North, Central, and South America. Some forty described species of the genus *Empoasca* are now known to occur in Mexico, thirty of which have been described in previous *Empoasca* studies in this series. Six new species are being described at this time. The types are in the author's collection.

Empoasca blendens n. sp.

A small blunt headed species resembling *aspersa* in general form but with unique coloration and markings. Length 3 mm.

Vertex: Bluntly angled, a little wider between eyes at base than median length.

Color: Vertex pale with orange markings which form a pale wedge shaped spot at apex with a round pale spot on the margin at either side. The basal portion is pale with an elongated orange mark on either side at base. Pronotum pale with a pair of broad longitudinal bands which are proximal on caudal half. They are bent outwardly and are more strongly separated on the anterior portion. Scutellum dark brown to black, with three longitudinal white marks on posterior half. Elytra pale with tips smoky. Most of the elytra are covered with yellowish blotches. A darker pigmented transverse band crosses at about the middle. This is composed of a dark reddish brown band on the clavus and bright reddish spots on the corium. The costal margin is usually yellow. Face pale with orange arcs.

Genitalia: Female seventh sternite broadly roundedly produced. This is a common species in several areas of Mexico but the male has not been obtained. It is described because of its unique coloration and appearance.

Holotype female and paratype females from Mexico City D.F., Sept. 1, 1939 collected by D. M. DeLong; and from the same locality Sept. 20, 1923 by Dr. Dampf. Female Paratypes from Tehuacan, Pue, Oct. 17, 1941 and from Puebla, Pue, Oct. 18, 1941 by DeLong, Good, Caldwell, and Plummer. Female paratypes from Desierto de Los Leones D.F., Dec. 4, 1927 by Dr. Dampf.

Empoasca jalapa n. sp.

A broad headed species resembling *cylpeata* in general appearance but with distinct male genitalia. Length 4 mm.

Vertex: Short and broadly rounded in front, appearing almost parallel margined, almost twice as wide between the eyes at base as median length.

Color: Pale to dark brown without definite markings. The elytra usually appearing darker, smoky.

Genitalia: Male styles, slender, elongate, tapering on apical half and pointed at apex. Lateral processes long, slender, and tapered to pointed apices. Aedeagus simple in form with a blunt apex. The pygofer spine is short and broad with a sharp pointed apex which is directed anteriorly.

Holotype male collected at Puebla, Pue (K-78) Oct. 18, 1941 by DeLong, Good, Plummer, and Caldwell. Male paratypes from Cuernavaca, Mor., Sept. 8, 1939 (Plummer and DeLong) and Sept. 25, 1945 (Plummer, DeLong, Hershberger, and Elliott); Mexico City D.F. Sept. 26, 1945 and Oct. 22, 1945 (DeLong, Hershberger, and Elliott); Jalapa Rd Vera, K-241, Oct. 13, 1945 (Shaw, DeLong, Hershberger, and Elliott); San Jacinto D.F. June 7, 1933 (M.F. 3040); and Bella Vista Mex. (M.B. 366) Sept. 17, 1933 by Dr. Dampf.

Empoasca cruciata n. sp.

A broad headed species, white with a brown cruciate marking on middle of elytra, resembling a *Typhlocyba* in general appearance. Length 4 mm.

Vertex: Broad, rather blunt, slightly produced at apex and rounded. Vertex narrower than pronotum. Almost twice as wide between eyes at base as median length.

Color: White, ocelli darker. Pronotum white, the dark anterior portion of the scutellum showing through from above. Scutellum dark brown with a narrow median pale longitudinal line. Elytra more heavily marked in male than in female specimens. In each sex the elytra are white with a dark brown cruciate band crossing the apical half of the clavus. The posterior arm of the cross extends diagonally, caudally to the costal margin anterior to the apical cells. The anterior arm is shorter and extends onto the corium (in the female) just anterior to the claval vein. In the male it extends across the corium to the costal margin. Also in the male there are brown markings on the elytra along the margin of the scutellum and along the commissural line to form a spot on middle of clavus. Face white with a dark brown spot on clypeus. Female ovipositor dark brown.

Genitalia: Female seventh sternite with posterior margin broadly roundedly produced. Male styles rather short with apices curved outwardly and pointed. The lateral processes are long, curved outwardly on apical fourth with the apices rather abruptly pointed. The aedeagus is rather straight, erect and slightly thickened at apex. The pygofer spine is rather broad extending caudally then curving ventrally with the pointed apex extending ventrally.

Holotype male, allotype female and male paratypes from Desierto de Los Leones D.F. Oct. 9, 1945 collected by DeLong, Hershberger, and Elliott. Male paratype from same locality (M.F. 1744) Aug. 24, 1930 collected by Dr. A. Dampf.

Empoasca angustella n. sp.

An elongate, blunt headed species, resembling *obstipa* in general appearance but with distinct genitalia. Length 4 mm.

Vertex: Broad, slightly produced and rounded. About two-thirds as wide between eyes at base as median length.

Color: Pale yellowish to orange. Vertex and anterior portion of pronotum yellowish. There are three white spots on anterior margin of pronotum. The posterior portion of the pronotum and scutellum is orange. There is a broad median, longitudinal white stripe on scutellum, darker margined, which is visible under pronotum. Elytra subhyaline tinged with yellow.

Genitalia: Male styles slender and with tapered sharply pointed apices. The lateral processes are rather short and thickened at the base. The aedeagus is erect and the apical portion is Y-shaped with the caudal branch longer and narrowed. The pygofer spine is rather short, and broadened on caudo-ventral portion. The anterio-ventral margin is sharp pointed.

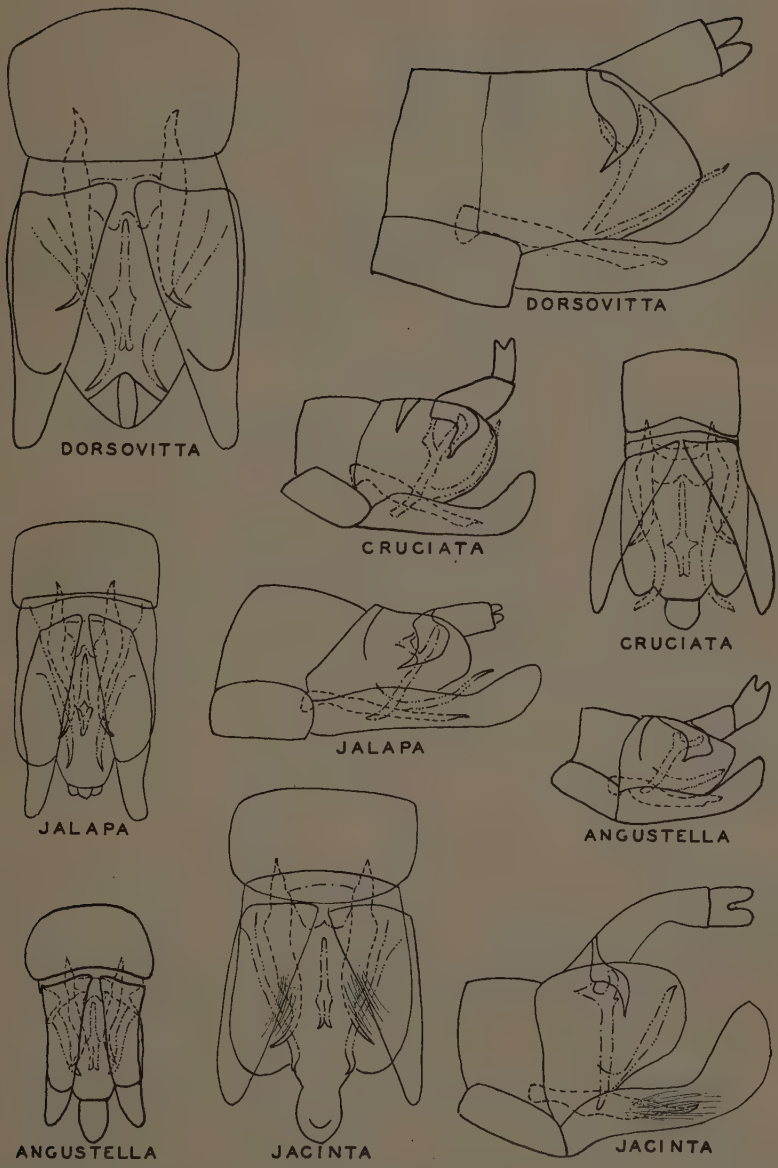
Holotype male collected at Laguna de Zempoala, Mor. Mexico, Oct. 21, 1945 collected by DeLong, Plummer, Hershberger, and Elliott.

Empoasca dorsovitta n. sp.

A small species resembling *cerata* in general form with a more pointed head and a dark colored stripe from apex of head to tip of wings. Length 3 mm.

Vertex: Produced and blunt at apex, almost two-thirds as long at middle as basal width between the eyes.

Color: Green, a broad black or dark brown stripe extends from apex of vertex to tips of wings. This is broadened from narrow stripe at apex to the width between the eyes at the base of vertex and covers the entire scutellum. There is a distinct notch in the band on either side at the anterior margins of the eyes. The stripe on the wings is brownish and covers most of the claval area. This merges with a smoky area covering the apices of the wings and extending anterior to the apex of the clavus. There is also a darker oblique band extending from the disc of the wing to the smoky area just anterior to the clavus. In female specimens the color is reduced on the wings to an elongate dark brown spot on each side of the scutellum on the base of the clavus and a dark elongate spot on the middle of each wing. The apex is smoky as in the male.



The figures are illustrations of the ventral and lateral views of the male genital structures.

Genitalia: Female seventh sternite broadly, roundedly produced, male styles elongate pointed at base and apex. The caudal portion is tapered to a slender pointed apex which is curved outwardly. The lateral pygofer processes are similar in shape to the styles with pointed apices which curve outwardly. In lateral view it is a simple straight process which gradually tapers to a pointed apex. The aedeagus is erect with an anterior process which arises a short distance from the apex, is rather long, tapered, sharp pointed and curving dorsally. The apical portion of the adeagus is thickened and rounded. The apical caudal spine is rather long, thickened to near the end then narrowed on the inner margin by a concave excavation, and is sharply pointed.

Holotype male, allotype female and female paratypes collected at Laguna de Zempoala, Mor., Oct. 21, 1945 by Plummer, DeLong, Hershberger, and Elliott; female paratypes from Zitacuara, Mich., Sept. 28, 1945 by the same collectors and Oct. 29, 1941 by DeLong, Good, Caldwell, and Plummer; Carapan, Mich., Oct. 21, 1941 by the same collectors; male and female paratypes from Rio, Frio D.F. (K-67) Oct. 18, 1915 by DeLong, Hershberger, and Elliott, and a male paratype from Cruz Blanca, Vera (K-289), Oct. 13, 1945 by Shaw, DeLong, Hershberger, and Elliott.

Empoasca jacinta n. sp.

Resembling *obtusa* in general appearance but with distinct male genital structures. Length 4.5 mm.

Vertex: Broadly rounded in front, almost transverse, about as long at middle as next the eyes, twice as wide between eyes at base as median length.

Color: Green tinged with yellow or orange, with paler areas but without definite markings. Elytra greenish subhyaline, often with a black spot on inner anterior margin of each eye.

Genitalia: Male styles rather broad, the apical third abruptly narrowed and tapered to a slender pointed apex. Lateral processes of the pygofers narrow, elongate, the apices slightly recurved and sharply pointed. The aedeagus is thickened and blunt at the apex. The dorsal pygofer spine is somewhat elongate, curved caudo-ventrally and sharp pointed at the apex.

Holotype male and male paratypes from San Jacinto D. F. (M.F. 3090) June 7, 1933 and Xochimilco D.F., Sept. 30, 1923 by Dr. A. Dampf.

The Life of Vertebrates. *J. Z. Young.* Oxford University Press, London. First edition, 1950. xv+767 pp. $8.50.

The main theme of this book appears to be basically that of vertebrate evolution. Each major vertebrate class is treated in order with special consideration to structural developments which resulted in the ancestral and extant forms, as well as those structures which were to lead ultimately to still higher groups. In so far as space and information permit, pertinent ecological information is introduced in an interesting and informative manner. More than a quarter of the text deals with the orders of Mammals and their lives of evolution and structural specialization.

To date, this represents the finest book of its kind since "Parker and Haswell." As yet no American equivalent in the field of Vertebrate Zoology and Morphology, either as a readable text-reference or as a source of widely diversified information concerning this important group of animals has appeared. The text material is both informative and understandable, and in addition very well illustrated. The evolution charts which cover each group, except for the birds, are as clear as the concise classification for each group.

This book would serve exceptionally well as a text for a real course in Comparative Vertebrate Anatomy (not for a pre-medical course), Vertebrate Zoology, and for an introductory course in Vertebrate Palaeontology. In addition, it will constitute an important adjunct to readings in Mammalogy, and could be used as a text in such a course along with supplemental readings.

FRED H. GLENNY.

SEASONAL AND HORMONE INDUCED CHANGES IN THE TESTES OF *RANA PIPIENS*

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The general conclusions of the majority of those biologists who have experimented with the frog *Rana pipiens pipiens* (Schreber) indicate that the male frog is an excellent test animal for the early diagnosis of human pregnancy. Nevertheless there are some unanswered questions which affect the use of this test. One of these is the possibility of seasonal variation in sensitivity in the frog, a period of time when the test animal is less responsive to chorionic gonadotrophin than in other seasons. This period of lessened sensitivity occurs during the summer months. It is important to determine the reason for this seasonal sensitivity since during this time false negative results for pregnancy tests are most frequently reported.

A histological study has been made of seasonal differences in the maturation and release of spermatozoa by *R. pipiens* after injections of concentrated pregnancy urine and injections of fresh anterior lobes of frog pituitary glands. The results are compared with the seasonal picture of testes of treated frogs. A study of the condition of the testes during the various seasons of the year has helped to answer the question of why there is seasonal variation in sensitivity of this species.

The purposes of this study were (a) to determine the variations in seasonal sensitivity of the male frog, *R. pipiens*, to 2.5 cc injections of pregnancy urine concentrate, (b) to compare the testes of frogs injected with pregnancy urine with testes of uninjected frogs, (c) to compare the testes of both pregnancy urine injected and uninjected frogs with frogs that have been injected with fresh anterior lobes of pituitary glands, and (d) to determine if the use of pregnancy urine concentrate during the summer months might be used as a safeguard against false negative results. To many using the test in the past, seasonal variation in sensitivity has appeared as quantitative variations in the number of mature spermatozoa per microscope field. The basis for this should be apparent in a histological examination of the testes, demonstrating the location and number of mature spermatozoa.

In 1948, Wiltberger and Miller first presented the use of *R. pipiens* as a new test animal for diagnosis of early pregnancy and suggested the possibility of seasonal differences in the reactions of frogs. Most investigators, with the exception of Cutler (1949) and Marsters, Black, and Randall (1950) report the male frog *R. pipiens* to be less sensitive during the summer months as indicated by quantitative estimates. Therefore, during August, when sensitivity of this species appears to be the lowest, another species, *Rana clamitans*, was compared with it in respect to the condition of the testes to determine if *R. clamitans* may be employed during August. This is the breeding season for *R. clamitans* in northern states. It also was necessary to determine whether cyclic spermatogenesis is the sole cause of reduced reactiveness in summer, as compared to other seasons of the year.

EXPERIMENTAL PROCEDURE

First morning urine specimens of a woman in her first trimester of pregnancy were collected daily during March, April and May, and stored in pint jars in a refrigerator at 10° C. These urine specimens were used in all of the experiments which were performed during that year. Apparently, the chorionic gonadotrophic hormone of pregnancy urine retains its potency for at least a year *if kept in a refrigerator at 10° C.* This observation indicates that the testing of a urine sample

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immediately after voiding is not necessary for accurate clinical results. However, before each experiment was performed the urine was checked for potency on a test frog.

Male *R. pipiens* weighing 30 to 35 gm were received from Vermont on the first day of every month. Upon arrival they were placed in a refrigerator at 10° C in about one and one-half inches of water. The frogs were held in the refrigerator for four or five days before being used. A testis was removed from a normal uninjected frog and fixed for sectioning to be used as a check.

The frog to be operated on was etherized lightly and placed on a clean towel. A small incision was made through the skin on the left ventral abdominal surface, at the border of the spotted area and anterior to the hip. The muscle of the body wall was pierced and torn with tweezers to the size of the slit made in the skin. The fat bodies and the left testis were located. The mesorchium was cut and the left testis removed and placed in Bouin's solution for fixation. In handling the testis was held by the mesorchium so that it would not be mutilated in any way, since, according to Rugh (1939) manipulation of the testis may force the release of some spermatozoa into the seminiferous tubules. This was considered a normal unstimulated testis. It is believed that the testis removed from an experimental animal before injection of the hormone serves as a better control than the testis from a different animal. With fine silk thread and a small surgical needle the muscle layer of the body wall and skin layer were closed. The frog was then placed in a one-quart jar at the bottom of which there was sterile cotton moistened with Ringer's solution.

After approximately one and one-half hours, when the operated frog had recovered activity, 2.5 cc of pregnancy urine concentrate were injected into the dorsal lymph sac. The frog remained in the jar at room temperature, occasionally being moistened with small amounts of Ringer's solution. After three hours the frog was again etherized and the right testis removed and fixed. To check for possible individual variation, in each case three frogs were operated on in this manner and the results compared.

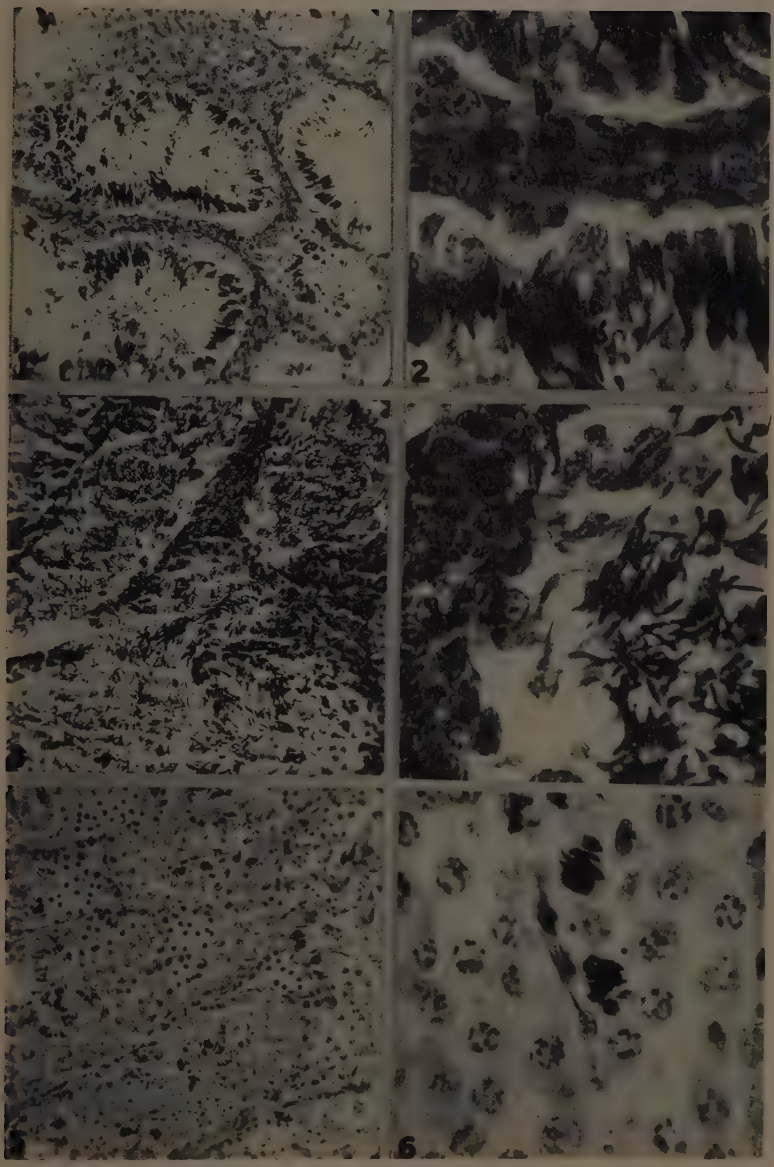
In order to determine how early the effect of the gonadotrophins could be detected in sections of testes, two sets of experimental animals were run and their testes removed at one-half hour, one hour and two hours.

To compare the effects of chorionic gonadotrophins with gonadtrophins of the pituitary four anterior lobes of male *R. pipiens* pituitary glands were injected into the dorsal lymph sac of frogs during each month and the results compared.

EXPLANATION OF FIGURES IN PLATE I

1. Sectioned testis of the uninjected frog in March. Several seminiferous tubules with spermatozoa clustered at the periphery.
2. Sectioned testis of the uninjected frog in March. A portion of two tubules of #1 under oil immersion.
3. Sectioned testis of the frog in March after injection of pregnancy urine concentrate. Several tubules with spermatozoa scattered freely in the lumen.
4. Sectioned testis of the frog in March after injection of pregnancy urine concentrate. A portion of #3 under oil immersion.
5. Sectioned testis of the uninjected frog in August. Lumen of the seminiferous tubules completely filled with maturation stages, and spermatozoa in clusters.
6. Sectioned testis of the uninjected frog in August. An enlarged portion of one seminiferous tubule with maturation stages and clumped spermatozoa.

All material illustrated was prepared from *Rana pipiens*, except where indicated, and was fixed in alcoholic Bouin's picro-formol solution, sectioned at 10 microns, and stained with Delafield's hematoxylin and eosin.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RESULTS

Condition of testes prior to the breeding season. In March, the sectioned testes of uninjected frogs show spermatozoa in clusters outlining the periphery of the seminiferous tubules (figs. 1, 2). Spermatogonial cells, primary and secondary spermatocytes, and spermatid cysts are rare. The germinal layers are either absent or one or two layers may be present, along with spermatogonial cells in some tubules.

The histological picture of the testes of frogs in March after an injection of known pregnancy urine concentrate shows spermatozoa within the lumen of the seminiferous tubules (figs. 3, 4). The spermatozoa are scattered freely, and are not found in groups or clusters as in the sections of uninjected frog testes. There is a definite disturbance in the testicular picture of frogs that had been injected with pregnancy urine

Condition of testes during the breeding season. April and May are the months when *R. pipiens* normally breeds in northern states. The breeding season varies depending upon the latitude and upon variations in the temperature. The histological picture of the testis of one uninjected frog in April, for example, shows nearly all spermatozoa on the periphery of the tubules. The spermatozoa are found closely clustered into groups. Stages of spermatogenesis are rare and therefore there are only one or two germinal layers present.

After stimulating the frog by means of an injection of pregnancy urine concentrate during the breeding season, three-fourths of the spermatozoa are released and discharged into the lumen. Some are clumped together into groups which are found in the lumen. Such clumps are often seen in the frogs urine during a pregnancy test. Not all tubules are affected equally. There may be some tubules with no scattered spermatozoa. In these cases the spermatozoa are clustered at the periphery of the tubules.

Condition of testes after the breeding season. The sectional testes of uninjected frogs in June and July show some spermatozoa still present which are located at the periphery of the tubules. Frogs injected with pregnancy urine concentrate in June and July show a picture similar to those frogs that were injected during March, April, and May with the exception that fewer spermatozoa are present. In August very few spermatozoa are found (figs. 5, 6). The tubule is almost completely filled with maturation stages, especially with numerous early prophase 1 stages. In August there are four to eight germinal layers in comparison to the two

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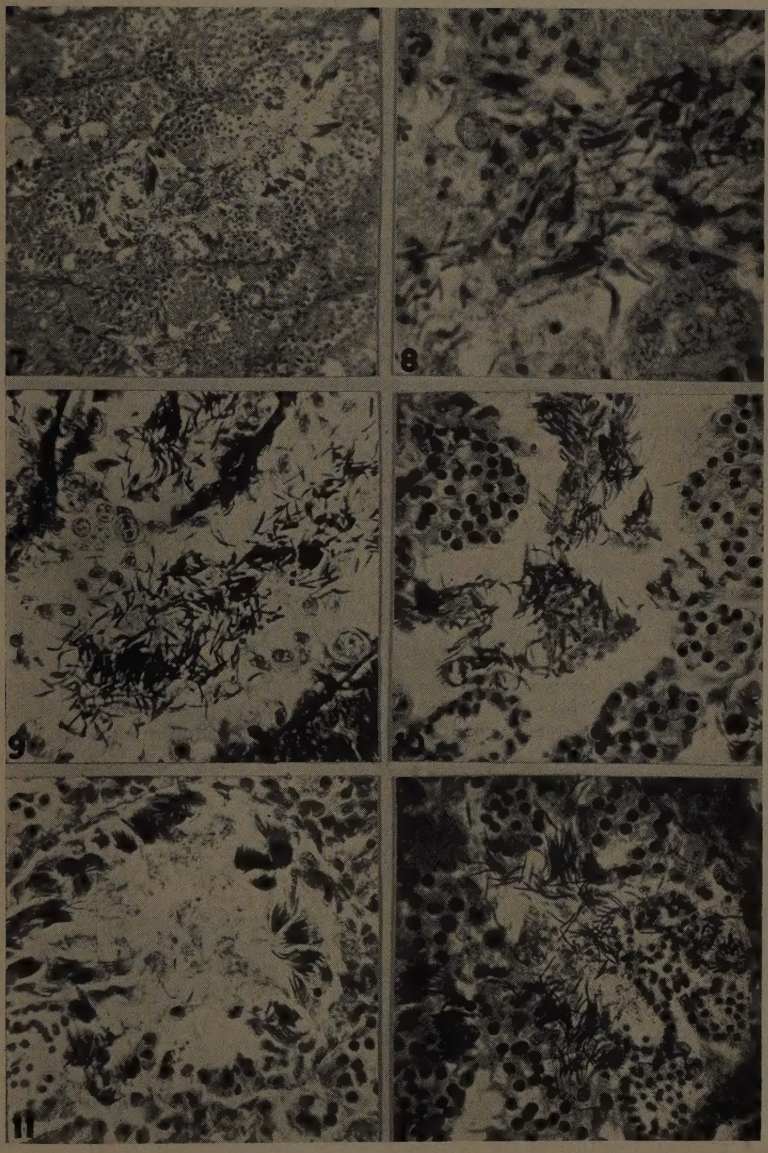
EXPLANATION OF FIGURES IN PLATE II

7. Sectioned testis of the frog in August after injection of pregnancy urine concentrate. Several tubules with the lumen filled with maturation stages; scattered spermatozoa, with some still grouped.
8. Sectioned testis of the frog in August after injection of pregnancy urine concentrate. An enlarged portion of a seminiferous tubule with stages of maturation, and scattered spermatozoa.
9. Sectioned testis of the frog in March after injection of four anterior lobes of frog pituitary glands. Spermatozoa are scattered freely in the lumen.
10. Sectioned testis of *R. clamitans* in August after injection of four anterior lobes of frog pituitary glands. Spermatozoa are scattered in the lumen.
11. Sectioned testis of uninjected *R. clamitans* in August. Spermatozoa are clustered near the periphery of the tubules.
12. Sectioned testis of *R. clamitans* in August after injection of pregnancy urine concentrate. Abundant spermatozoa and spermatid stages.

All material illustrated was prepared from *Rana pipiens*, except where indicated, and was fixed in alcoholic Bouin's picro-formol solution, sectioned at 10 microns, and stained with Delafield hematoxylin and eosin.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or three germinal layers in April and May. After injections in August a smaller number of spermatozoa were found as compared to other months. Practically the entire tubule was filled with cells undergoing maturation divisions (figs. 7, 8).

Condition of testes before the hibernation season. September and October can be considered the pre-hibernating period in *R. pipiens*. Sections of testes of uninjected frogs in October differ from those in August in that there are more spermatozoa present, more late maturation stages are present, and there is a noticeable increase in the size of the lumen of the tubules. The testes sectioned after injections of pregnancy urine concentrate show results similar to those described for March.

Condition of testes during the hibernation season. The histological picture of the testes of uninjected hibernating frogs during November, December, January, and February appears similar to the testes of uninjected frogs in March. The spermatozoa are abundant during the frogs hibernation period and are attached to the border of the seminiferous tubules. There are one to three germinal layers present including secondary spermatocyte and spermatid stages. Frogs injected with pregnancy urine concentrate during these months show the characteristic testicular picture.

During November and February, testes had been removed from frogs after intervals of one-half hour, one hour, and two hours following the injection of pregnancy urine concentrate. In all cases the left testis of the uninjected frog was compared to the right testis of the same frog after an injection of pregnancy urine concentrate. In all cases there was an apparent stimulation.

Collectively these results are consistent in that all the frogs stimulated either with pregnancy urine concentrate or anterior lobes of pituitary glands (figs. 9, 10) demonstrated the pronounced effect of the release of spermatozoa into the seminiferous tubules.

In August the sectioned testis of uninjected *R. pipiens* was compared with the sectioned testis of uninjected *R. clamitans* (fig. 11). It is during this month that the responsiveness of *R. pipiens* to injection of pregnancy urine appear to be most depressed. In sectioned testes of *R. clamitans* the spermatozoa are clustered and grouped together at the periphery of the tubules. There are some maturation stages and many tubules have an abundance of spermatid stages. In sectioned testes of *R. pipiens* there are fewer spermatozoa and maturation stages almost completely close the lumen of the tubules. After injections of pregnancy urine concentrate the sectioned testes of *R. clamitans* (fig. 12) shows abundant release of spermatozoa.

From the histological evidence it seems apparent that during the summer months cyclic spermatogenesis is the cause of lessened reactiveness of the male frog, *R. pipiens*, to injections of pregnancy urine concentrate. It also appears that in *R. clamitans* more spermatozoa are present and therefore there is a greater number of spermatozoa released after the injections.

Photomicrographs of sectioned testes after pregnancy urine injections and after injections of fresh anterior lobes of pituitary glands during all seasons of the year can be found in a dissertation by Biesinger (1951) in The Ohio State University library.

CONCLUSIONS

1. A study of the sectioned testes of the uninjected male frog, *R. pipiens*, discloses cyclic variations, indicating seasonal changes. The histological appearance of the testes of the uninjected frog prior to the breeding season in March (figs. 1, 2) and during the breeding season (April, May) shows spermatozoa in clusters and closely grouped together at the periphery of the seminiferous tubules.

2. The sectioned testes of frogs injected with pregnancy urine concentrate show a definite testicular change since after the injections there is a release of spermatozoa into the lumen of the tubules (figs. 3, 4, 7, 8, 12).

3. The effects of injections of pregnancy urine concentrate are similar to the effects of injections of anterior lobes of pituitary glands (figs. 9, 10).

4. From the histological results it seems apparent that during the summer months, cyclic spermatogenesis is the cause of reduced sensitivity of the male frog, *R. pipiens* to injections of pregnancy urine concentrate (figs. 5, 6). This lessened activity suggests the advisability of using concentrate of pregnancy urine for pregnancy tests as a safeguard against false negative results, especially during the post-breeding season.

5. During August, the sectioned testes of uninjected *R. clamitans* (fig. 11) show a larger number of spermatozoa attached to the basement membrane of the tubules than found in *R. pipiens* (figs. 5, 6). It may be suggested that *R. clamitans*, if it is available, would make a satisfactory substitute for *R. pipiens* during this month.

6. There is no apparent difference in the testicular picture due to time lapse after the pregnancy urine injections. Frogs allowed to stand for one-half hour, one hour, two hours, and three hours were not essentially different.

7. The chorionic gonadotrophic hormone of pregnancy urine retains its potency for at least one year if stored in a refrigerator at 10° C. This observation indicates that the testing of a urine sample soon after voiding is not necessary for accurate clinical diagnosis.

8. Since the chorionic gonadotrophic hormone does not seem to deteriorate readily, samples of pregnancy urine may be stored and used for demonstrations, as for producing live spermatozoa for classroom purposes.

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A Concise History of Astronomy. *Peter Doig.* Philosophical Library, Inc., New York. First edition, 1951. xi+320 pp. $4.75.

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